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Immunoassayable Somatomedin C in seminal plasma of azoospermic men

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Summary

Somatomedin C is a Sertoli cell peptide and since measurements of other Sertoli cell products in semen have provided a useful indices of testicular function, it was considered pertinent to measure the semen levels of Somatomedin C. Somatomedin C was measured by RIA in seminal plasma of vasectomized subjects ($n = 18$), subjects with agenesis of the seminal vesicles and vasa deferentia ($n = 6$) and subjects with azoospermia resulting from seminiferous tubule damage without obstruction ($n = 23$). Normal fertile subjects (24 men with a sperm concentration $> 20 \times 10^6$/ml) were used as controls. In all subjects, seminal levels of transferrin were also measured as an index of Sertoli cell function. The majority of seminal Somatomedin C appears to derive from the testis and/or epididymis. However, in several normal controls seminal levels of Somatomedin C (median = 3.52; range = 1.10–15.67 U/ejaculate) were found to be within the range for vasectomized subjects (median = 0.78; range = 0.46–4.20 U/ejaculate). In subjects with azoospermia the seminal levels of Somatomedin C (median = 2.06; range = 0.60–10.12 U/ejaculate) were significantly lower ($P < 0.02$) than in fertile controls. However, values for these two groups overlapped. It is concluded that Somatomedin C in semen is not a reliable index of seminiferous tubule function and does not appear to be of diagnostic value in male infertility.

Keywords: Seminal Somatomedin C, seminal transferrin, azoospermia.

Introduction

The Sertoli cells secrete many proteins, some of which are specific for the testis, such as inhibin and androgen–binding protein (ABP) and others, which are similar or identical to serum proteins, such as transferrin, somatomedins, ceruloplasmin and plasminogen activator (Mather *et al.*, 1983; Holmes, Lipshultz & Smith, 1984). These proteins can be measured in human seminal plasma and may, therefore be of possible clinical value as indices of seminiferous tubule function.

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Previous reports from this (Orlando *et al.*, 1985, Caldini *et al.*, 1986) and other laboratories (Holmes, Lipshultz & Smith, 1982) have demonstrated that, in man, 80% of seminal transferrin derives from the Sertoli cell. Moreover, seminal transferrin is below the normal range in patients with azoospermia resulting from severe seminiferous tubule damage without obstruction. In the same studies (Orlando *et al.*, 1985, Caldini *et al.*, 1986) a significant correlation was found between seminal transferrin levels and sperm number. Therefore, seminal transferrin may be a useful clinical index of seminiferous tubule function.

Until now, Somatomedin C has not been measured in seminal plasma from azoospermic men. In this paper we report values for seminal Somatomedin C in men with azoospermia of various aetiologies. These results have been compared to values for seminal transferrin in the same subjects.

Patients and methods

Patients

Azoospermic patients derived from three groups: (a) vasectomized subjects, at least 1 year after operation ($n = 18$); (b) men with agenesis of the vasa deferentia and seminal vesicles as demonstrated by semen analysis and transrectal echography ($n = 6$); (c) subjects with azoospermia due to seminiferous tubule damage as demonstrated by testicular biopsy, performed according to a previously described procedure (Forti *et al.*, 1981) ($n = 23$). The latter subjects were assumed to be without obstruction on the basis of the seminal levels of free L-carnitine (Soufir, Marson & Jouannet, 1981) and epididymo-vesiculodeferentography. This radiological investigation was performed during anesthesia for testicular biopsy by injection of 5 ml Uromiro 240 (an aqueous solution of iodamide in 45% meglumine and 55% sodium hydroxide, corresponding to 420 mg/ml iodine; Bracco, Italy) into the vasa deferentia using a 27 gauge needle for lymphangiography.

A group of 24 normal fertile subjects with sperm concentrations of 20×10^6/ml were used as controls. All groups were matched for age.

Methods

In all subjects, testicular volume was measured using a Prader orchidometer. Semen collection and analysis were performed according to World Health Organization recommended procedures (WHO, 1980), after 4 days of sexual abstinence. Seminal plasma was separated by centrifugation at $2500 \times g$ for 10 min and stored at -20°C until assayed.

Somatomedin C was measured using reagents (rabbit anti-serum to human Somatomedin C, Somatomedin C standard and iodinated tracer) produced by the Nichols Institute (San Juan Capistrano, CA, U.S.A.). The human Somatomedin C was purified from Cohn fraction IV-4 according to the procedure described by Svoboda *et al.*, (1980). The assay procedure was as follows. Somatomedin C standard (5–200 mU/ml) or seminal plasma samples (diluted 1:20 with assay buffer) were pre-incubated with anti-serum for 1 h at room temperature. After addition of the iodinated tracer (about 10 000 c.p.m./tube), the tubes were incubated overnight at 4°C. Separation of bound and free was performed using a second antibody raised in goats.

In the same seminal plasma samples, transferrin was measured using a chemiluminescent immunoassay (Caldini *et al.*, 1986).

Serum FSH level was measured in all subjects by an RIA method (Forti *et al.*, 1981) and in the same serum samples transferrin and Somatomedin C were also measured.

Seminal free L-carnitine was measured by a radioenzymatic method (Boehmer, Rydning & Solberg, 1974; McGarry & Foster, 1976).

Statistical analysis

All data are presented as the median and range. Comparisons between groups were made using Wilcoxon's test for unpaired data.

Results

The within- and between-assay coefficients of variation (CV) for Somatomedin C in human seminal plasma were 5.2% ($n = 15$) and 9.0% ($n = 15$), respectively. Different dilutions of seminal plasma showed linearity with the Somatomedin C standard over the range 0.62–10 μl/tube ($y = 0.997x + 0.05$; $r = 0.994$; $n = 40$). Recovery tests ($n = 8$) performed by adding different doses of standard Somatomedin C (10, 25, 50 mU) to a constant volume of seminal fluid, showed a mean recovery of 108±7.0% (mean ± SD).

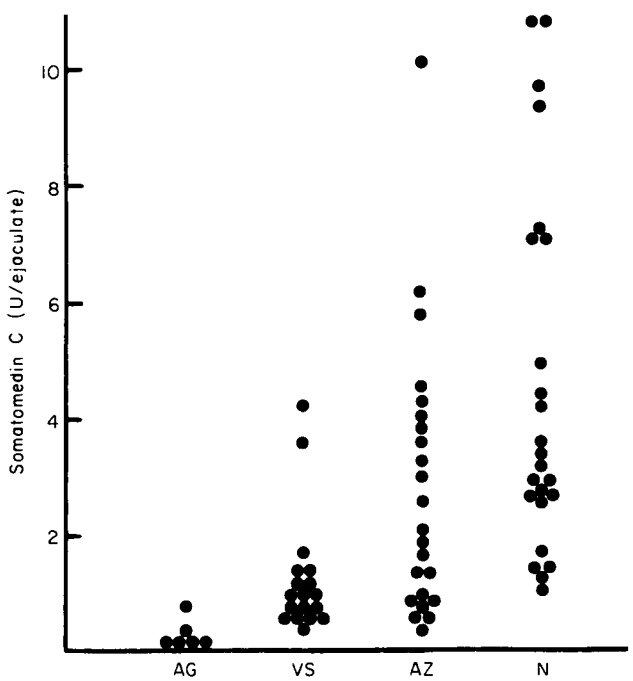

Fig. 1. Levels of immunoreactive Somatomedin C in seminal plasma (expressed as Units per ejaculate) from subjects with agenesis of the vasa deferentia and seminal vesicles (AG; $n = 6$) (median + range = 0.37; 0.09–1.00), vasectomized patients (VS; $n = 18$) (1.00; 0.38–4.20), subjects with azoospermia due to seminiferous tubule damage (AZ; $n = 23$) (2.06; 0.60–10.12) and normal fertile controls (N; $n = 24$) (3.52; 1.10–15.67).

The anti-serum used in the assay was highly specific for Somatomedin C and showed 30% cross-reactivity with Somatomedin A, 1% cross-reactivity with IGF-II and $<0.1\%$ cross-reactivity with insulin, LH, FSH, TSH, hGH, epidermal growth factor, IGF I-C peptide, ovalbumin, lactoferrin and transferrin.

No difference was found between the Somatomedin C concentration measured in freshly collected samples (3.18±1.13 U/ml; mean ± SD) and after storage at -20°C (3.34±1.42 U/ml; mean ± SD) for at least 2 months ($n = 10$). Data were evaluated by the paired Student's *t*-test. The intra-individual variability calculated in four subjects (five samples each) was 5.9% (CV).

Values for seminal Somatomedin C in the four groups of subjects are illustrated in Fig. 1. Seminal levels of transferrin in the same subjects are illustrated in Fig. 2. The median value for serum Somatomedin C and transferrin in subjects with azoospermia resulting from seminiferous tubule damage were 1.03 (range: 0.48-2.04) U/ml and 2171 (range: 1953–3584) μg/ml, respectively, while, in normal controls the corresponding values were 1.11 (range: 0.61–2.38) U/ml and 2190 (range: 2010–3761) μg/ml, respectively. Serum levels of FSH were 25.0 (range: 6.4–57.3) mIU/ml in azoospermic patients and 6.8 (range: 4.5–12.1) mIU/ml in normal controls.

In azoospermic patients and normal controls no correlation was found between the seminal levels of Somatomedin C or transferrin, serum FSH and testicular volume. Moreover, no correlation was found in normal controls between seminal

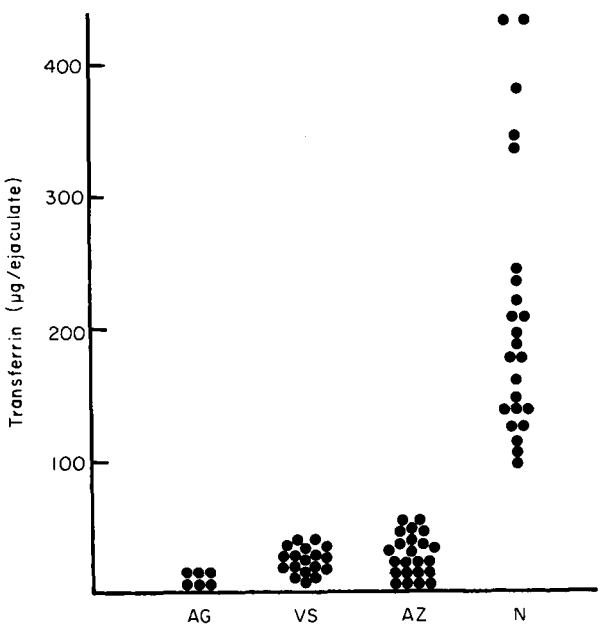

Fig. 2. Levels of transferrin in seminal plasma (expressed as μg per ejaculate) from subjects with agenesis of the vasa deferentia and seminal vesicles (AG; $n = 6$) (median + range = 3.8; 3.0–16.0), vasectomized patients (VS; $n = 18$) (7.7; 2.6–40.3), subjects with azoospermia due to seminiferous tubule damage (AZ; $n = 23$) (27.5; 5.9–70.3) and normal fertile controls (N; $n = 24$) (180.6; 100.1–532.4).

Somatomedin C and the total sperm count. A weak positive correlation was found between seminal transferrin and the total sperm count ($r = 0.573$; $P < 0.01$).

Discussion

Mean values for seminal Somatomedin C found in this study were comparable to levels in blood. However, the median value for seminal Somatomedin C found in vasectomized patients (0.78; range 0.46–4.20 U/ejaculate) was only about 25% of the median values found in normal fertile controls ($P < 0.001$). This suggests that the majority of seminal Somatomedin C is testicular and/or epididymal in origin. In several normal controls Somatomedin C values were found to be in the range of vasectomized subjects, while in the same subjects, seminal transferrin values were always above the range found in vasectomized patients (Figs 1 and 2). In subjects with azoospermia resulting from seminiferous tubule damage the Somatomedin C median value was significantly lower than in fertile controls ($P < 0.02$), but there was overlap between values for the two groups. In contrast, these groups could be discriminated easily by the measurement of seminal transferrin (Figs 1 and 2).

The relative contribution of the seminiferous tubules and epididymis to Somatomedin C concentrations in human seminal plasma is unknown. In addition, it is not known if the immunoassayable Somatomedin C measured in human seminal plasma is identical to that which circulates in the blood. With respect to the origin of Somatomedin C in seminal plasma of vasectomized subjects, the comparison of median values with those in patients with agenesis of the seminal vesicles and vasa deferentia (0.15, range 0.19–0.43 U/ejaculate) ($P < 0.001$) clearly demonstrates that the contribution from prostatic secretions is small.

In conclusion, if values for vasectomized and normal patients are compared, it appears that the majority of immunoassayble Somatomedin C in semen derives from the testis and/or epididymis. However, seminal Somatomedin C does not appear to be as reliable an index of seminiferous tubule function as is seminal transferrin when applied to the diagnosis of male infertility.

Acknowledgments

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