Immunologically reactive albumin-like protein in human testis and seminal plasma*

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Summary. An immunologically reactive albumin-like protein (albumin) was localized, by an immunostaining technique, in the testis of infertile men (normal spermatogenesis, obstructive azoospermia) at the level of the Sertoli cells and in some cells of the germinal epithelium (secondary spermatocytes and early spermatids). No positive reaction was detectable in prepubertal testis.

In vasectomized men, mean seminal albumin values were drastically reduced (by about 80%) in comparison to fertile controls, indicating a probable testicular origin. Mean seminal albumin values were also decreased in patients affected by azoospermia due to a seminiferous tubular lesion (about 40%) and in oligozoospermic patients (about 30%). In the same seminal samples transferrin, an index of Sertoli cell function, was also measured. Albumin and transferrin results were well correlated in the seminal plasma of each group (with the exception of vasectomized subjects), including a group of men with abnormally high concentrations of seminal transferrin. A weak correlation was found between seminal albumin and sperm count. We suggest that the presence of albumin in the human adult testis and in seminal plasma could be related to its ability to transport androgens.

Keywords: albumin; transferrin; human seminal plasma; Sertoli cell proteins

Introduction

Human semen contains many protein components which are probably important to provide a suitable environment for the survival and function of spermatozoa. Some of these proteins are specifically secreted by male accessory glands (acid phosphatase by the prostate) or by the epididymis (α -1,4-glucosidase) (Casano *et al.*, 1987). Several other proteins are produced by the Sertoli cell and can be divided into testis-specific (inhibin, ABP) and serum-like (e.g. transferrin, somatomedin-C, ceruloplasmin, plasminogen activator) proteins (Mather *et al.*, 1983). Finally, a specific isoenzyme of lactate dehydrogenase (LDH-X) is released by germinal cells of the post-pubertal testis (Blanco & Zinkman, 1963). All these proteins have been extensively studied in human semen in an attempt to determine their physiological role and clinical relevance in evaluation of male fertility.

Besides these 'organ-specific' proteins, human seminal plasma is rich in other proteins, most of which have been clearly identified with electrophoretic techniques (Quinlivan, 1968; Edwards *et al.*, 1981): albumin, lactoferrin, IgA, IgG, fibrinogen, prealbumin, trypsin and several other enzymes. For some of them, their origin as well as their role inside the male genital tract remain to be elucidated.

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Tauber *et al.* (1975) demonstrated that seminal albumin and transferrin are present in higher concentrations in the first parts of the ejaculate. Bardin *et al.* (1986) have reported that rat Sertoli cells in culture synthesize a protein which is immunologically and structurally related to serum albumin and which has been termed 'testibumin'. 'Testibumin' secretion is increased both by testosterone and FSH. Testicular and epididymal concentrations of this protein increase in rats from birth to puberty.

In this paper we report the results of immunohistochemical localization of an albumin-like protein (albumin) in the testis of pre-pubertal boys and adult men with normal or abnormal spermatogenesis and the results of albumin measurement, by a very sensitive chemiluminescent method, in seminal plasma of patients affected by infertility of different origin and in normal fertile subjects. In the same samples seminal transferrin, an index of Sertoli cell function *in vivo* (Orlando *et al.*, 1985), was also measured.

The individual spontaneous variation in time of seminal albumin levels is also reported.

Materials and Methods

Immunostaining of albumin

Patients. Testicular biopsies of adult men (26–36 years of age) were obtained under epidural anaesthesia from patients (N = 8) being investigated for obstructive azoospermia. Only men with normal spermatogenesis were considered. Testicular biopsies were obtained from 2 prepubertal boys during orchidopexy for undescended testis; in both boys there was macroscopic evidence of disjunction between the testis and epididymis.

A total of 300 sections were studied; 80 of them were used as control sections.

Reagents and procedure. Albumin (A 8763), rabbit antiserum against human albumin (A 8273) and goat anti-rabbit IgG peroxidase conjugate (A 8275) were obtained from Sigma Chemical Company, St Louis, MO, U.S.A. Diaminobenzidine (3,4,3',4'-tetraaminodiphenylhydrochloride) was obtained from BDH Chemicals Ltd (Poole, Dorset, U.K.).

Testicular specimens were fixed in Bouin's solution and processed by peroxidase–antiperoxidase immunocytochemistry (Sternberger, 1979). Sections were first incubated with antiserum against human albumin (titre 1:400 in 0.1 mM-PBS, pH 7.4) and then with the second antibody–peroxidase conjugate (titre 1:1000). Peroxidase activity was revealed by a 0.225 mM solution of diaminobenzidine in 0.05 mM-Tris-HCl buffer containing 0.01% H₂O₂.

Positive results were checked by serial dilution of specific immune serum (from 1:800 to 1:8000), using the second antibody–peroxidase conjugate alone, incubating with non-immune serum and by preabsorption of specific antiserum with an excess of human albumin.

Albumin assay in seminal plasma

Patients. The subjects investigated in the first part of the study were divided into four groups: (a) vasectomized subjects, at least 1 year after surgery (N = 7); (b) patients affected by azoospermia due to seminiferous tubular damage (N = 39) proven by testicular biopsy; (c) oligozoospermic men (N = 69) with a sperm count of $<20 \times 10^6$ /ml and $<100 \times 10^6$ /ejaculate; (d) a group of 9 subjects (3 azoospermic, 3 oligozoospermic and 3 normospermic men, without evidence of haemospermia, confirmed by cytological examination of seminal sediments) who had shown abnormally very high levels of seminal transferrin ($>99\%$ confidence limit of the respective sub-group range: mean ± 3 s.d.). Normal fertile subjects (N = 71) were used as controls.

In the second part of the study 5 fertile male volunteers (30–35 years of age) were investigated to examine the spontaneous variation of the substances measured in semen in this study. They provided semen samples at regular intervals (29 days minimum–32 days maximum) after a fixed sexual abstinence period of 3 days, yielding 12 specimens over 1 year. They were free of any disease during the study period and the administration of any drug was avoided. They were not chosen on the basis of the quality of the semen.

Materials and procedures. Semen collection and analysis were performed according to the World Health Organization recommended procedure (Aitken *et al.*, 1987). Routine examination of semen specimens was always performed by the same technician. All semen samples were collected in our laboratory by masturbation, after 3 days of sexual abstinence, and centrifuged after liquefaction at 1000 g for 15 min at +4°C. The supernatant was removed and stored at –20°C for albumin and transferrin assay (within 1 week).

Seminal transferrin and albumin were measured by immunochemiluminescent methods previously described (Caldini *et al.*, 1986; Orlando *et al.*, 1987). Human albumin and antialbumin antiserum were the same as those used for the immunohistochemical study. ABENH (7-[(N-4-aminobutyl)-N-ethylamino]naphthalene-1,2-dicarboxylic acid hydrazide (Miles Co., Naperville, IL, U.S.A.) was used as the tracer. Preparation and purification of the tracer were as described elsewhere (Messerli *et al.*, 1986).

The intra- and inter-assay coefficients of variation (calculated on 12 different determinations) were respectively 5.1% and 8.5% for seminal albumin and 7.8% and 9.3% for seminal transferrin determinations. In our laboratory the coefficient of variation of sperm count determinations, performed by the same technician, and assessed in 12 different aliquants of the same semen sample, ranges from 15 to 21%.

Statistical analysis. Statistical analysis of the results was performed by Student's *t* test and regression analysis of linear correlation. Only the results expressed per total volume of ejaculate were statistically analysed because the total amount of the biochemical measures is a more reliable index than their relative seminal concentrations, which are the result of dilution in the fluids originated by seminal vesicles and prostate.

Results

Immunohistochemical study

A clear immunoreactivity for albumin was obtained in Sertoli cells of normal seminiferous tubules (Fig. 1) as well as in those showing different degrees of tubular damage. Positive staining was also evident in all seminiferous tubules at the level of secondary spermatocytes and early spermatids. There was no positive staining in any control sections (Fig. 2). No immunological reaction was present in seminiferous tubules of the prepubertal testes (Fig. 3).

Fig. 1. Section of normal seminiferous tubule treated with antiserum to human albumin (1:400 dilution). Immunoreactive albumin is localized in the cytoplasm of Sertoli cell (S) and in many round cells, interpreted as secondary spermatocytes (SC) and early spermatids (ES). $\times 320$.

Fig. 2. Control section incubated with non-immune serum. No positive reaction is detectable. $\times 320$.

Albumin assay

As shown in Table 1, in vasectomized patients there was a strong and identical decrease (about 80% of normal controls) in values of albumin and transferrin in semen. Seminal values were also

Fig. 3. Section from prepubertal testis. No specific reactivity is detectable (1:400 antiserum dilution). $\times 430$.

Table 1. Seminal values (mean $\pm$ s.e.) of albumin and transferrin in infertile patients and normal controls

	Albumin		Transferrin		
	$\mu\text{g}/\text{vol.}$	%	$\mu\text{g}/\text{vol.}$	%	$\text{ng}/\mu\text{g albumin}$
Fertile (N = 71)	2700.0 ± 146.3	100	196.0 ± 14.4	100	75.7 ± 4.2
Oligozoospermic (N = 69)	1924.7* ± 133.5	71.3	89.3* ± 10.3	45.6	45.4* ± 4.2
Azoospermic (N = 39)	1605.4* ± 142.9	59.5	58.4* ± 9.5	29.8	37.6* ± 4.5
Vasectomized (N = 7)	531.9* ± 88.1	19.7	35.6* ± 10.9	18.2	78.6 ± 32.7
Abnormally high transferrin (N = 9)	10 244.0* ± 1318.0	379.4	690.4* ± 97.3	352.1	69.8 ± 10.0

*Significantly different from normal controls ($P < 0.001$).

significantly decreased in oligozoospermic men and in patients affected by azoospermia due to seminiferous tubular damage, although the decrease of transferrin values was more evident. In men with abnormally very high concentrations of seminal transferrin there was an identical increase in albumin seminal values (about 3.5-fold). When expressed as micrograms of albumin, seminal transferrin values were still reduced in oligozoospermic and azoospermic patients, while in vasectomized men and in subjects with abnormally high concentrations of seminal transferrin the values were similar to those of normal controls.

A significant correlation was found between seminal albumin and transferrin in each group ($r = 0.6112$, $P < 0.001$ for fertile donors, $r = 0.5683$, $P < 0.001$ for oligozoospermic men; $r = 0.5312$, $P < 0.001$ for azoospermic men; and $r = 0.7064$, $P < 0.005$ for subjects with abnormally high levels of seminal transferrin) with the exception of vasectomized subjects ($r = -0.0545$, not significant). When all subjects were considered, a very high correlation was found ($r = 0.8371$, $P < 0.001$). A weak correlation ($r = 0.4244$, $P < 0.001$) was found between albumin and total

sperm number; such a correlation was not dissimilar that found between transferrin and sperm count ($r = 0.4814$, $P < 0.001$).

In the study performed on 5 male volunteers to determine the intra-individual variability of seminal albumin and transferrin values, the mean variability (% coefficient of variation) of seminal albumin (mean: 26.92%; range: 11.47–36.75%) was not different from that of transferrin (mean: 27.52%; range: 13.70–38.31%) and evidently lower than that of sperm count (mean: 44.31%; range: 32.28–55.56%). In this study albumin correlated well with transferrin values ($r = 0.5254$, $P < 0.001$); the correlation with sperm count was very poor even if statistically significant ($r = 0.3748$, $P < 0.01$).

Discussion

Our findings suggest the presence of an immunologically reactive albumin-like protein in Sertoli cells, in secondary spermatocytes and early spermatids of men with normal spermatogenesis. These results seem to be in agreement with the reported hormonally dependent secretion of a protein structurally and immunologically related to serum albumin (called 'testibumin') (Bardin *et al.*, 1986). Immunohistochemical localization revealed that this protein in rats is mainly localized in Sertoli cells and in epididymal epithelium. In addition, the absence of a positive reaction in the human prepubertal testis is in agreement with similar results found in prepubertal rats (Bardin *et al.*, 1986).

The results of the immunohistochemical localization of albumin in the human testis are similar to those obtained by us (Vannelli *et al.*, 1986) for transferrin, a specific iron-carrier protein and an index of Sertoli cell function. According to those results, obtained using a specific monoclonal antibody to transferrin receptors (Vannelli *et al.*, 1986), we suggest that the positive reaction for albumin at the level of secondary spermatocytes and early spermatids could be explained by the presence of a complex albumin–albumin receptor in these cells. The presence of a specific and saturable receptor for albumin has been demonstrated (Brandes *et al.*, 1982; Ockner *et al.*, 1983) in several types of cells (hepatocytes, adipocytes, erythrocytes). This receptor could act by promoting the uptake into target cells of substances bound to albumin (Szego, 1975; Siiteri *et al.*, 1982; Ockner *et al.*, 1983). We therefore suggest that, in addition to androgen binding protein, at the level of seminiferous tubules albumin could also act as a carrier for androgens. Cumming & Wall (1985) and Manni *et al.* (1985) have suggested that albumin-bound testosterone as well as free testosterone can readily enter cells because of the short time of dissociation of a testosterone–albumin complex (Baird *et al.*, 1969; Pardridge, 1981). Such an hypothesis was also advanced to explain the presence of histoimmunochimically reactive albumin at the level of granulosa cells (Spanel-Borowski, 1987).

The results we obtained for immunochemically reactive albumin in the semen of vasectomized men suggest that the testis is the principal source of seminal albumin, as previously demonstrated for transferrin (Orlando *et al.*, 1985; Caldini *et al.*, 1986). The decrease in seminal values of albumin in oligozoospermic and azoospermic patients was less marked than that found for transferrin, suggesting that the secretion of albumin is a process that is less sensitive to Sertoli cell impairment.

In subjects with abnormally high values of seminal transferrin without evident haemospermia, we found a parallel and marked increase of albumin-like protein concentrations. Such a phenomenon could be explained by an increased permeability of the male genital tract to serum proteins. This hypothesis is supported by the finding of a seminal albumin/transferrin ratio similar to that of serum in these subjects. Furthermore, when expressed in terms of micrograms of albumin, seminal transferrin values were still reduced in oligozoospermic and azoospermic patients in comparison to normal controls. Similarities in the patterns of these two proteins are confirmed by the similar correlation with sperm count and by the identical intra-individual variability over time.

In conclusion, our results demonstrate the presence of a protein, immunologically and structurally related to serum albumin, at the level of the seminiferous tubules of human adult testis. The

physiological role of this protein is not yet known. However, we suggest that this 'testicular albumin' could act as a carrier of androgens in the tubular compartment.

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