Rapid decline of fertility in a case of adrenoleukodystrophy

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Adrenoleukodystrophy/adrenomyeloneuropathy (ALD/AMN) is a group of genetically determined peroxisomal disorders associated with progressive central demyelination, primary adrenal cortical insufficiency (Addison’s disease) and, frequently, primary hypogonadism. Recently, testicular dysfunction was described in ALD/AMN patients but no information on sperm characteristics was provided. In this paper we studied the reproductive function of a patient with adult cerebral ALD, focusing our attention on sperm characteristics. At the time of diagnosis the patient was 22 years old, had high plasma C26 and C24 very-long-chain fatty acid (VLCFA) concentrations and adrenal insufficiency. Plasma testosterone concentration was in the normal range. The patient was prescribed a low-fat diet and ‘Lorenzo’s oil’, which led to normalization of plasma VLCFA concentrations within 3 months of therapy. Semen analysis showed normal sperm count, gross morphological alterations and reduced motility. Electron microscopy analysis of sperm cells showed pathological changes in the head, the plasma membrane and the nucleus in 60% of the spermatozoa examined. However, isolated motile spermatozoa showed normal molecular dynamics of phospholipid bilayer surface and physiological responsiveness to progesterone. At the 12 months follow-up, the patient became azoospermic and testicular histology showed arrested maturation. To our knowledge, this is the first description of sperm alterations in a post-pubertal ALD patient, in which severe impairment of spermatogenesis and rapid progression to azoospermia occurred despite normalization of plasma VLCFA concentrations.

Key words: Addison’s disease/central demyelination/hypogonadism/sperm membrane/testosterone

Introduction

Adrenoleukodystrophy (ALD) is an X-linked disease affecting 1/20 000 males whose biochemical defect is characterized by impaired β-oxidation of very-long-chain fatty acids (VLCFA) in peroxisomes, particularly hexacosanoic acid (C26:0), pentacosanoic acid (C25:0), and tetracosanoic acid (C24:0), which accumulate in tissues and body fluids (Moser et al., 1991). The ALD gene, mapped to Xq28, has recently been cloned (Mosser et al., 1993) and was shown to encode for a peroxisomal transporter protein which may be involved in the activation of the VLCFA-coenzyme A synthetase, and whose activity is deficient in ALD. The phenotype of ALD is varied, and at least seven clinical subtypes have been described: childhood cerebral ALD (the more severe form), adolescent cerebral ALD, adult cerebral ALD, adrenomyeloneuropathy (AMN), Addison’s disease only, and presymptomatic (asymptomatic) and heterozygous women (Moser et al., 1991). Adrenal insufficiency (Addison’s disease) is frequently associated with ALD/AMN (Auborg and Chausain, 1991); however, primary hypogonadism has been reported more frequently during AMN, but not ALD (Maris et al., 1995). To our knowledge, there is no information on semen analyses in ALD/AMN patients.

We report a patient with adult cerebral ALD presenting with normozoospermia that rapidly evolved to azoospermia. Sterility appeared despite normalization of VLCFA concentration in blood obtained after ‘Lorenzo’s oil’ was administered. Electron microscopy studies on sperm morphology and evaluation of molecular dynamics of sperm phospholipid bilayer membrane are also presented.

Case report

Case history and physical recordings

A 22 year old male subject was referred to our institution in June 1996 because of weakness of the lower limbs and gait disturbances. In the family history his mother’s brother died in his forties of severe neurological disturbances. The medical history revealed that during his military duties, when he was 19 years old, he complained of mild asthenia and cutaneous hyperpigmentation. At the time of hospitalization, the physical examination showed the presence of an intense generalized hyperpigmentation of the skin surface (particularly the scrotum) and the buccal mucosa; male-pattern baldness was present and pubic and axillary hair were scarce. Testicular size was slightly reduced (15 ml bilaterally). Neurological examination revealed moderate ataxia and spastic paraparesis, without signs of visual, auditory, or peripheral nerve dysfunction. Body mass index (BMI) was normal (21) and blood pressure was 105/75 mm Hg.
Endocrine and biochemical investigations

After giving an informed consent the subject underwent endocrine investigations and evaluation of plasma VLCFA. Hormonal and VLCFA determinations were performed according to procedures published elsewhere (Fabbri et al., 1988; Fraioli et al., 1989; Laureti et al., 1996). Results showed the presence of high adrenocorticotropic hormone (ACTH) (6100, 6800 and 6200 pg/ml at 0800, 1400 and 2000 h; normal values 20–100) and low cortisol (35, 47 and 44 ng/ml; normal values 50–250), low dihydroepiandrosterone sulphate (DHEAS) (0.6 pg/ml; normal values 0.8–5.6) and 17-hydroxy-progesterone (108 pg/ml, normal values < 200) plasma concentrations. 24 h urinary free cortisol was low (4 μg/24 h; normal values 10–90). Supine plasma renin activity and aldosterone concentrations were slightly above the normal range (23 pg/ml, normal values 0.3–20.2, and 16.3 pg/ml, normal values 3–15, respectively) and did not increase after the upright postural test (26 pg/ml and 11.4 pg/ml, respectively). No concomitant electrolyte alterations were found (sodium 142 mEq/l, normal values 135–144, potassium 3.4 mEq/l, normal values 3.6–5.4). ACTH testing (Synacthen 250 μg i.v.) showed no response of Δ-4-5 adrenal steroids and cortisol (Table I). Basal thyroid stimulating hormone (TSH) and follicle stimulating hormone (FSH) were slightly above the normal range, while luteinizing hormone (LH) concentrations were 2.5 times the upper limit of normal (Table I). Basal- and reverse-T3, free-T4, TSH, testosteron, free testosteron and prolactin were within the normal range. Thyrotropin releasing hormone [TRH (200 μg i.v.)] + gonadotrophin-releasing hormone (GnRH) stimulation (100 μg i.v.) produced a brisk increase in serum TSH and LH, and lesser increase in serum FSH and prolactin (PRL). Human chorionic gonadotrophin (HCG) stimulation (10 000 UI i.m.) revealed no testosterone response at all time points examined (24, 48, 72 and 96 h after stimulus) (Table I). Plasma antibody concentrations for thyroglobulin, microsomes, thyroid peroxidase, thyroid receptor, gastric mucosa, glutamic acid decarboxylase and 21-hydroxylase were absent. The analysis of fatty acids of plasma sphingomyelin (Laureti et al., 1995) showed the presence of 40% double heads, 55% disrupted membranes and 65% abnormalities of both nuclei and nuclear matrix (Table II). The number of spermatozoa free from defects in the total ejaculate was very low [110×10^9, normal values > 2×10^10 according to Baccetti’s formula (Baccetti et al., 1995)]. Intracellular calcium concentration ([Ca^{2+}]) and membrane fluidity were evaluated by Fura2 and Laurdan fluorescence spectroscopy, respectively (Palleschi and Silvestroni, 1996; Silvestroni et al., 1997). These investigations were carried out with motile, viable spermatozoa isolated by a ‘swim-up’ procedure (Vijayakumar et al., 1987). All the patient’s data were compared with those obtained in contemporaneous studies with spermatozoa from normozoospermic, fertile donors (n > 20; WHO Guidelines). Both basal [Ca^{2+}] and membrane fluidity were in the normal ranges, with the latter being at the upper limit of the prediction interval (Figure 1, left panel). Sperm challenging with prostaglandin E1 produced a steep increase of [Ca^{2+}], (Figure 1, right panel), which was similar to controls (Figure 2, inset) and testified a physiological sperm responsiveness to the agonist.

Therapy and follow-up

The patient was prescribed a low-fat diet and received daily doses of glycerol trioleate oil (1.7 g/kg of body weight) and glycerol trirucinate (0.3 g/kg) (Lorenzo’s oil) (Aubourg et al., 1993) along with hydrocortisone 37.5 mg/day. After 3 months of therapy with Lorenzo’s oil, the patient’s plasma concentrations of VLCFA were reduced to the normal range (C24 from 66.34 to 40.4 μmol/l; C26 from 2.559 to 0.556 μmol/l) and remained normal during the 6, 9 and 12 month follow-up periods (C24 = 41.9 μmol/l; C26 = 0.550 μmol/l at 12 months). Platelet number decreased from 300×10^3 to 80×10^3 as a direct consequence of the therapy, as previously described (Aubourg et al., 1993). However, the sperm number decreased

**Table I. Basal and dynamic hormonal recordings in a patient with adult cerebral adrenoleukodystrophy (ALD)**

<table>
<thead>
<tr>
<th>Test</th>
<th>Hormones</th>
<th>Basal</th>
<th>Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH</td>
<td>FSH (1–9 mU/ml IRMA)</td>
<td>12.2</td>
<td>16.4</td>
</tr>
<tr>
<td>TRH</td>
<td>TSH (0.2–4.0 mU/ml IRMA)</td>
<td>6.4</td>
<td>26.1</td>
</tr>
<tr>
<td>HCG</td>
<td>testosterone (2.8–9 ng/ml RIA)</td>
<td>5.5</td>
<td>5.4</td>
</tr>
<tr>
<td>ACTH</td>
<td>cortisol (50–250 ng/ml RIA)</td>
<td>62</td>
<td>63</td>
</tr>
<tr>
<td>DHEA sulphate (0.8–5.6 pg/ml RIA)</td>
<td>0.6</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>17-hydroxyprogesterone (&lt; 200 pg/ml RIA)</td>
<td>108</td>
<td>59</td>
<td></td>
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GnRH = gonadotrophin-releasing hormone; TRH = thyrotrophin-releasing hormone; HCG = human chorionic gonadotrophin; ACTH = adrenocorticotropic hormone; FSH = follicle stimulating hormone; LH = luteinizing hormone; TSH = thyroid stimulating hormone; PRL = prolactin; DHEA = dihydroxyandrostenedione; IRMA = immuno-radiometric assay; RIA = radioimmunoassay.
Figure 1. Left panel: temperature-dependence of Laurdan excitation generalized polarization (GPex) values obtained at 340 nm (upper traces) and 410 nm (lower traces) excitation wavelengths. Symbols indicate GPex values of spermatozoa from adrenoleukodystrophy (ALD) patient. Mean GPex values (dashed lines) with relative prediction intervals (dotted lines) of spermatozoa from fertile, healthy donors are also shown. Right panel: effect of progesterone addition (2 µg/ml, arrow) on intracellular free calcium ([Ca^{2+}]_i nM) in Fura2-loaded ALD spermatozoa. Inset: typical response of spermatozoa from control subjects.

<table>
<thead>
<tr>
<th>Number</th>
<th>Velocity</th>
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<tbody>
<tr>
<td>50 × 10^6/ml; total ejaculate = 5.0 ml</td>
<td>speed: 54.3 ± 22.5 (mean ± SD) µm/s (44.7 ± 20.1)</td>
</tr>
<tr>
<td>(≥ 20×10^6 ml)</td>
<td>linear speed: 23.0 ± 10.3 µm/s (28.7 ± 16.7)</td>
</tr>
<tr>
<td>Motility</td>
<td>linearity: 4.4 ± 1.7 (6.1 ± 2.3)</td>
</tr>
<tr>
<td>40% after 30 min</td>
<td>mean LHA: 2.7 ± 1.2 µm (1.7 ± 0.9)</td>
</tr>
<tr>
<td>(≥ 60% rapidly progressive)</td>
<td>max LHA: 6.1 ± 2.5 µm (4.7 ± 2.5)</td>
</tr>
<tr>
<td>BCF: 11.2 ± 2.9 cycles/s (11.9 ± 2.9)</td>
<td></td>
</tr>
</tbody>
</table>

Morphology (light microscopy)
typical forms = 35% (≥ 60%)
immature forms = 3%
atypical forms = 62% (< 40%)
(presence of head abnormalities, double heads, swelling tails)
observations: physiological fluidification, normal viscosity, pH = 7.3, 1 × 10^6 leukocytes

Morphology (electron microscopy)
state of spermatozoa 40% simple
40% double
20% multiple
plasma membrane 45% integral
55% broken
acrosome 40% present
60% absent
nucleus shape: 35% normal
65% abnormal
chromatin: 55% homogeneous
45% margnated (uncondensed)
mitochondria helix assembly: 55% regular
45% irregular
shape: 55% regular
45% swollen
cytoplasmic residues 60% present
40% absent
axoneme pattern: normal 65%
altered 35%
arms: normal 70%
lacking 30%
shape: normal 60%
rolled up 40%
spermatozoa devoid of defects*: 110 × 10^6 (>2 × 10^6)

LHA = amplitude of lateral head displacement; BCF = beat cross frequency.
*From Baccetti et al. (1995).
Sperm alterations in ALD

Figure 2 A, B. Seminiferous tubules are dilated and hypocellular with arrested maturation. Germ cells are decreased and represented by spermatogonia, rare spermatocytes and early spermatids. The interstitium contains a foamy, proteinaceous fluid and Leydig cell clusters [A, haematoxylin–eosin original magnification (OM) ×200; B, haematoxylin–orange-phosphomolybdic acid-aniline blue (HOPA) OM ×200, (Fabbrini, 1953)]. C. Preparation of testicular tissue to show basal membranes which do not appear thickened. Sertoli cells are also evident (PAS OM ×200). D. Higher magnification of seminiferous tubules showing Sertoli cells only (arrowheads) and depletion of germ cells. Microvacuolation of Sertoli cells is clearly seen. Fragmentation is discernible in Leydig cells (arrows) (haematoxylin–eosin OM ×400). Bar (panels A, B, C) = 50 µ; bar (panel D) = 25 µ. Pr: proteinaceous fluid; ST: seminiferous tubule.

progressively during the 3 (38×10^6/ml), 6 (30×10^6/ml), 9 (8×10^6/ml) and 12 (azoospermia) months follow-up periods respectively. A testicular biopsy was performed after azoosper- mia appeared and testis histology showed similar aspects in both testes. The seminiferous tubules appeared atrophic and dilated (Figure 2), with conserved aspect of the basal membrane. The germ cell population was significantly decreased and was mainly represented by spermatogonia, rare spermatocytes and, in some tubules, early spermatids. Furthermore, the cytoplasm of all germ cells showed degenerative alterations along with aspects of microvacuolations of Sertoli cells (Figure 2). In the interstitium, the number of Leydig cell clusters per seminiferous tubule, determined with a standard Zeiss calibrated grid and the 25× objective lens (Weiss et al., 1979; Powers and Schaumburg, 1981), was reduced, compared with controls (0.36 versus 0.8–1.1). Also, Leydig cells showed both cytoplasmic microvacuolations and fragmentation (Figure 2). Neurophysiological studies repeated at 12 months confirmed
a significant deterioration of neurological function, which in turn resulted in a clinical condition of complete spastic paraparesis.

Discussion

Before the diagnosis of ALD, the patient was admitted to the hospital with the suspicion of idiopathic Addison’s disease. The presence of a multiple autoimmune endocrine disorder was excluded on the basis of the absence of antibodies against adrenal, thyroid and pancreatic glands. Multiple sclerosis was excluded by a clinical history of progressive neurological disturbances, normal cerebrospinal fluid (CSF), and the peculiar magnetic resonance imaging (MRI) picture. In Schilder’s disease (diffuse cerebral sclerosis), the neurological disorders and the cerebral lesion are often similar to those of ALD, but adrenal atrophy is unique to the latter. The finding of elevated plasma VLCFA was pathognomonic for ALD. AMN was ruled out because in AMN patients, MRI scans show demyelination in the spinal cord and the nerve conduction study shows a peripheral neuropathy, whereas in our patient both examinations were normal. Furthermore cranial MRI showed a large involvement of the white matter in the anterior portion of the brain and the cerebral peduncles. Although less frequent than the involvement of the posterior cerebral lobes, the MRI picture was characteristic of adult cerebral ALD. The finding of a prolonged conduction time along the corticospinal tracts, as demonstrated by motor-evoked potentials recording, was consistent with the cerebral peduncle demyelination shown by MRI scans. Although MRI scans did not show abnormalities in the acoustic and somatosensory pathways, both brainstem acoustic-evoked potentials and somatosensory-evoked potentials were delayed, as often found in patients with ALD (Garg et al., 1983; Laureti et al., 1996).

ALD may be a frequent cause of idiopathic Addison’s disease in children and adults (Blevins et al., 1994; Laureti et al., 1996), but endocrinological symptoms usually precede neurological symptoms in only 39% of patients with ALD and 61% of patients with AMN (Korenke et al., 1997). In accordance with other authors’ findings, our patient showed normal plasma renin activity and aldosterone concentrations with a conserved mineralocorticoid hormone economy (Cappa et al., 1990). The absent response of Δ4-Δ5 adrenal steroid pathways to ACTH stimulus is also consistent with primary adrenal insufficiency, as usually observed in ALD patients (Blevins et al., 1994). Thyroid function appeared to be slightly impaired, since elevated basal TSH concentrations and a brisk increase of TSH after TRH stimulation were found. The presence of a normal thyroid on ultrasound, normal plasma-free fraction of thyroid hormones and absence of thyroid microsomal antibodies suggested the presence of a euthyroid sick syndrome (Chopra, 1997).

Clinical and subclinical hypogonadism are rarely associated with ALD and it has been reported that testicular deficiency is more frequently associated with the presence of long-standing neurological abnormalities in AMN pubertal boys (Libber et al., 1986). Recent reports highlighted the presence of more frequent testicular involvement in a large group of ALD patients (Brennemann et al., 1997). In our patient, plasma testosterone concentrations were normal. However, the finding of slightly reduced bilateral testicular size, increased basal and GnRH-stimulated gonadotrophin concentrations (particularly LH) associated with an absent testosterone response to HCG test, indicated the presence of subclinical primary testicular dysfunction. This observation led to a deeper investigation of the fertility of the patient. Despite a normal number of ejaculated spermatozoa, we observed an altered sperm morphology, mainly consisting of abnormalities in the shape of the head and the plasma membrane integrity, as well as in the nuclear condensation (Baccetti et al., 1997). In contrast, axonal ultrastructure was normal. VLCFA play a major role in the plasticity of red blood cell membranes and it has been demonstrated that these cells exhibit altered biological functions in ALD subjects (Arietti et al., 1996). The plasma membrane of human spermatozoa also contain VLCFA, predominantly Δ-, Δ- and Δ-triene fatty acids with up to 32 carbon atoms (Poulos et al., 1986); however, the function of VLCFA in the plasticity of sperm structure and motility remains to be elucidated. In our patient, the plasma membrane of motile ALD spermatozoa exhibited both normal physical properties and responsiveness to physiological stimuli despite the presence of high plasma concentrations of VLCFA indicating that lipid membrane disorders in ALD patients are not homogeneously distributed among different tissues. To investigate this possibility additional studies should be conducted in the newly developed mouse model for ALD produced by targeted disruption (knock-out) of the peroxisomal fatty acyl-CoA gene (Fan et al., 1996).

Testicular histological findings have been described previously in ALD/AMN patients and consist of hypcellularity and mild vacuolation of seminiferous tubules, and interstitial damage – i.e. focal fibrosis near hyalinized tubules and reduction of the number of Leydig cell clusters per seminiferous tubule (Powers and Schaumburg, 1981). Testicular histology in our patient showed similar alterations, which mainly consisted of the presence of abundant proteinaceous fluid in the interstitium, reduction of Leydig cell number and germinal cell arrest. These pathological changes might explain the poor response of the testes to HCG observed in our, and other ALD/AMN, patients (Powers and Schaumburg, 1981). Indeed, a number of investigators have demonstrated that testicular damage associated with infertility is often associated with a subnormal response to HCG stimulation (De Kreter et al., 1975). In the tubules the damage involved both meiotic and maturative phases of spermatogenesis with arrest at the stage of early spermatids. Alterations of Sertoli cells consisted of microvacuulations, a histological aspect that has been reported in a variety of other testicular disorders [e.g. germinal aplasia (Chemes et al., 1977), essential fatty acid deficiency (Hildebrand-Stark and Fawcett, 1977)]. It is well known that the integrity of Sertoli cells is critical for the maintenance of adluminal germ cells and spermatogenesis (Gnnessi et al., 1997); therefore vacuolated Sertoli cells might be incapable of fulfilling their normal role of sustaining maturation of spermatocytes and spermatids, resulting in abnormal spermatids and death of differentiating germ cells. Indeed in our
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