Depletion of Alcohol (Hexanol) Dehydrogenase Activity in the Epidermis and Jejunal Mucosa in Sjögren-Larsson Syndrome

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Using a histochemical technique, we have demonstrated a consistent deficiency of alcohol (hexanol) dehydrogenase activity within the epidermis and jejunal mucosa of patients with Sjögren-Larsson syndrome. Biochemical assay of the fatty alcohol: NAD oxidoreductase activity in cultured fibroblasts and leukocytes from these patients showed deficient activities compared with controls.

The histochemical and biochemical results are complementary, and the simpler histochemical method can be used reliably for initial screening of patients with ichthyosis in whom a diagnosis of Sjögren-Larsson syndrome is suspected.

*J Invest Dermatol 95:632–634, 1990*

Sjögren-Larsson syndrome (SLS) is an inherited disorder, first described in 1957 [1], comprising ichthyosis, spastic di- or tetraplegia, and mental retardation. Clinically, the ichthyosis is seen at birth and initial presentation may be as a collodion baby. Signs of neurological disease generally become apparent after the age of three months with delayed developmental milestones and the onset of spasticity. The presence of glistening dots on the macula is regarded as pathognomonic for SLS although these are not present in all patients. In 1982, Hernell and co-workers [2] suggested that there was an abnormality of essential fatty acid metabolism in SLS. Subsequently, we identified abnormal essential fatty acid profiles in three patients with SLS*. Ample dietary fatty acid supplementation was ineffective, raising the possibility of a specific malabsorptive defect.

Recently, Rizzo and co-workers [3,4] reported deficient fatty alcohol: NAD oxidoreductase activity (FAO) in cultured fibroblasts and leukocytes of patients with SLS and a partial reduction in obligate carriers of the condition.

FAO is one of the family of alcohol dehydrogenases. It is partially inactivated by palmitoyl-CoA, and it is the palmitoyl-CoA-sensitive component that is reported to be most affected in SLS.

This work prompted us to investigate histochemically demonstrable alcohol dehydrogenase (AD) activity in skin and jejunal biopsies of patients with SLS.

**MATERIALS AND METHODS**

**Study Subjects**

Five girls (aged 14 months, 3, 8, 10, and 12 years) and two boys (aged 18 months and 4 years) with the typical clinical features of SLS were studied. They all exhibited generalized hyperkeratosis with marked involvement of the flexures and furrows radiating from the umbilicus. Five of the seven patients had the characteristic maculopatly, and all displayed moderate to severe mental retardation and spastic diplegia.

**Collection of Biopsies and Blood**

Skin biopsies (4-mm punch biopsies) were performed in all seven patients with local anaesthesia (1% lignocaine).

Per-oral jejunal biopsies were performed in three patients with the Watson paediatric capsule.

Five ml of venous blood was collected from each patient. Informed consent for each of these procedures was obtained from the parents.

Our control skin biopsies were from patients with non-bullous ichthyosiform erythroderma (three patients), X-linked ichthyosis (two patients), ichthyosis vulgaris (two patients), porokeratosis of Mibelli (one patient), hidrotic ectodermal dysplasia (one patient), graft-versus-host disease (one patient), dermatomyositis (one patient), and normal skin taken at the time of surgery for non-skin related conditions (ten patients).

Skin biopsies were divided into three portions: a) one part was fixed in buffered formalin for routine histology; b) one part was snap-frozen in hexane maintained at solid carbon dioxide temperature (−70°C), for histochemical examination, and c) one part was placed in tissue culture medium so that a fibroblast cell culture for subsequent biochemical assay could be established.

Jejunal biopsies were divided for routine histology, after buffered formalin fixation and paraffin embedding, and cryostat sections, after snap-freezing. Jejunal biopsies from patients being investigated for malabsorption were used as controls.

Blood was placed into sequestrene for leukocyte harvesting for the biochemical assay.

**Histology**

Sections for routine microscopy were stained with hematoxylin and eosin.

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Histochemical Demonstration of Alcohol Dehydrogenase

Cryostat sections (12 μm) of the snap-frozen skin and jejunal biopsies were air-dried, delipidized in cold (4°C) acetone for 5 min, and rapidly dried in a stream of air before incubation at 37°C for 90 min in the following medium: 0.1 ml 1 mol/l neutralized hexanol (BDH Ltd, Poole, Dorset, UK); 2 mg NAD (nicotinamide adenine dinucleotide) (Boehringer, Mannheim, Lewis, East Sussex, UK); 1 ml standard dehydrogenase medium. The pH was adjusted to 7.4 before use. The standard dehydrogenase medium, as described by Pearce [5], contains 5 ml 0.05 mol/l TRIS-HCL buffer, pH 7.4; 0.5 mg MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) (Sigma, Poole, Dorset, UK), in 5 ml distilled water; 0.1 ml 0.5 mol/l cobaltous chloride; 7 ml distilled water.

After incubation, the sections were washed in running tap water and mounted in glycerine jelly. A positive reaction was indicated by the deposition of blue/black formazan within the active cells. In other incubations, ethanol or octanol were substituted for hexanol, or the substrate was omitted.

Preparation of Cells for Biochemical Studies Skin fibroblasts from five patients were cultured by standard techniques in Hams F10 medium containing 15% fetal calf serum and antibiotics. As controls, we used skin fibroblasts from three subjects who had no history of skin or neurological disease. Trypsinized confluent cells were used for enzyme assays.

Leukocytes were separated from fresh venous blood of five patients with SLS and six control patients (age range, 2–62 years) with mental retardation and no history of skin disease. Erythrocytes were removed by hypotonic lysis in the presence of ammonium ions [6].

Fatty Alcohol:NAD Oxidoreductase Assay The enzyme assay on fibroblasts and leukocytes was carried out as described by Rizzo et al [4] on cultured fibroblasts and harvested peripheral blood mixed leukocytes, with [14C]-hexadecanol (24 mCi/mmol, obtained from Amersham International, Aylesbury, Bucks, UK) as substrate. The palmitoyl-CoA-sensitive component was measured in the presence of 80 μmol/l palmitoyl-CoA. The reference enzyme, β-galactosidase, was measured as described by Besley and Moss [7].

RESULTS

Histology Skin biopsies from the seven patients with SLS had similar histologic features: acanthosis, hyperkeratosis, and a prominent granular layer. Some also had papillomatosis. No other abnormal features were present. The jejunal biopsies were morphologically normal, and there was no lipid deposition, inflammatory infiltrate, or reduction in the villus/crypt ratio.

Histochemistry (Fig 1A-C) Alcohol dehydrogenase activity in the control skin biopsies was present in the keratinocytes and hair follicles and weakly in fibroblasts (Fig 1A, B). Most of the activity was in the basal layers, with none appearing above the granular layer. Activity was strongest with hexanol and negative with ethanol. The activity with octanol was weak, probably due to the poor solubility of this substrate in aqueous media. No reaction was obtained in the absence of substrate. Because hexanol gave the most intense reaction, it was used in all further incubations. The use of cold acetone for removal of lipids had no effect on the intensity of the reaction but gave a crisper reaction without the problem of lipid solubility of the formazan.

In the seven patients with SLS, there was a marked reduction or absence of activity in the epidermis (Fig 1C).

The normal jejunal enterocytes showed strong activity, whereas no activity was detected in the enterocytes of patients with SLS (Fig 1A, B).

Alcohol (hexanol) dehydrogenase activity was undetectable in leukocytes in blood films from normal subjects due to its low level of expression in these cells. Similar negative, or at best, weakly positive, results were obtained for normal fibroblasts grown on cover slips, thus precluding direct comparison with the biochemical assay.

Biochemistry (Tables I and II) All seven patients with SLS had markedly deficient FAO activity in fibroblasts and/or leukocytes. The deficiency was more marked when expressed as the palmitoyl-CoA-sensitive activity and was most evident in this study with cultured fibroblasts. All activities were assayed in duplicate, and values similar to those reported by Rizzo et al [3,4] were recorded. β-galactosidase activity, as a reference enzyme, was comparable in SLS and control subjects.
DISCUSSION

Lipids comprise the major component of the stratum corneum intercellular matrix and of the myelin sheath. It has now become recognized that many of the hereditary ichthyoses are associated with inborn errors of lipid metabolism. Abnormalities of essential fatty acid metabolism have been noted in SLS [2]. In 1988, Rizzo and co-workers [3] reported deficient FAO activity in cultured fibroblasts of patients with SLS. They demonstrated the accumulation of large amounts of a fatty alcohol (hexadecanol) in cultured fibroblasts, due to impaired oxidation of fatty alcohols to fatty acids. FAO is essential for the recycling of fatty alcohol-derived carbon chains by catalyzing the oxidation of fatty alcohols (such as hexadecanol and octadecanol) to their equivalent fatty acids. In normal cells, a component of FAO is sensitive to inhibition by palmitoyl-CoA. According to Rizzo et al [3], this component was most significantly decreased in SLS fibroblasts, being about 1% of normal. In their most recent paper [4], however, this residual activity is given as 10% of normal in fibroblasts and 15% of normal in leukocytes. Our findings are in agreement more with these later results.

We have shown that there is a depletion or absence of histochemically demonstrable alcohol dehydrogenase activity, with hexanol as a substrate, in the keratinocytes of patients with SLS and that the reduction of activity is sufficiently pronounced for a definitive diagnosis to be made. The results agree with those derived from the biochemical assay of FAO on cultured fibroblasts and leukocytes from the SLS patients.

In addition, we have shown that alcohol dehydrogenase activity is severely depleted in the enterocytes of jejunal biopsies from patients with SLS, thus confirming the generalized nature of the defect.

We conclude that the histochemical test described provides a useful and rapid method for the diagnosis of SLS. Further studies are in progress to investigate its potential for the detection of heterozygote carriers and for pre-natal diagnosis.

REFERENCES


Table I. Enzyme Activities in Fibroblasts from Patients with SLS and from Control Subjects

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total</th>
<th>Palmitoyl-CoA*</th>
<th>β-Galactosidase*</th>
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<td></td>
<td>Fatty Alcohol: NAD Oxidoreductase Activity (pmol/min per mg Protein)</td>
<td>Sensitive (mmol/min per mg Protein)</td>
<td>(mmol/min per mg Protein)</td>
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<tr>
<td>1</td>
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<td>3.44</td>
<td>8.63</td>
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<td>2</td>
<td>5.06</td>
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* Component inhibited by palmitoyl-CoA.
† Not done.