Clinical and biochemical findings in 7 patients with X-linked adrenoleukodystrophy treated with Lorenzo’s Oil

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Abstract

X-Linked adrenoleukodystrophy (X-ALD) is a hereditary disorder of the peroxisomal metabolism biochemically characterized by the accumulation of very long chain fatty acids (VLCFA) in tissues and biological fluids. The major accumulated acids are hexacosanoic acid (C₂₆:₀) and tetracosanoic acid (C₂₄:₀). The disorder is characterized clinically by central and peripheral demyelination and adrenal insufficiency closely related to the accumulation of fatty acids. The incidence of X-ALD is estimated to be 1:25,000 males. At least six phenotypes can be distinguished. The most common phenotypes are childhood cerebral ALD and adrenomyeloneuropathy (AMN). The recom-mended therapy consists of the use of the glyceroltrioleate/glyceroltrierucate (GTO/GTE) mixture, known as Lorenzo’s Oil, combined with a VLCFA-poor diet. There are alternative treatments such as bone marrow transplantation and immunosuppression, as well as the use of lovastatin and sodium phenylacetate. In the present study we report the clinical and biochemical course of 7 male patients with X-ALD treated with Lorenzo’s Oil and a VLCFA-restricted diet. Treatment produced 50% reduction in C₂₆:₀ and 42.8% reduction in the C₂₆:₀/C₂₄:₀ ratio. Most patients remained clinically well, although approximately 30% of them presented a rapid clinical deterioration. The results showed a poor biochemical-clinical correlation for treatment, indicating that new therapies for X-ALD are needed in order to obtain a better prognosis for patients.

INTRODUCTION

Adrenoleukodystrophy (X-ALD), the most common peroxisomal disease, is an X-linked metabolic disorder with an estimated frequency of 1:25,000 males (Wanders et al., 1993, 1995; Ruiz et al., 1996; Geel et al., 1997; Moser, 1997). Peroxisomes catalyze essential metabolic reactions (Wanders et al., 1993, 1995). Peroxisomal diseases can be classified into three groups according to the extent of loss of peroxisomal function: generalized, multiple or simple (Wanders et al., 1993, 1995; Geel et al., 1997). X-ALD is included in the last group since, in this case, peroxisomes are unable to perform β-oxidation (Wanders et al., 1995; Geel et al., 1997), resulting in impaired shortening of fatty acid chains and their derivatives (Wanders et al., 1993, 1995).

X-ALD is characterized biochemically by the accumulation of very long chain fatty acids (VLCFA) in tissues and biological fluids (Moser et al., 1994; Scriver et al., 1995; Moser, 1995, 1997). Mapping and isolation of the X-ALD gene have shown that the gene encodes a peroxisomal membrane protein, ALD protein (ALDP), which belongs to the ATP-bound carrier protein superfamily (Moser et al., 1994; Moser, 1995, 1997; Yamada et al., 1999). These observations indicated that this protein is somehow involved in the process of VLCFA oxidation (Moser et al., 1994; Moser, 1997).

X-ALD is clinically heterogeneous including different phenotypes (i.e., childhood cerebral form (ALD), juvenile cerebral form, adult cerebral form, adrenomyeloneuropathy (AMN), isolated Addison disease, and asymptomatic patients) (Moser et al., 1991; Scriver et al., 1995; Moser, 1995, 1997; Geel et al., 1997) reported within the same family (Moser et al., 1991, 1994; Scriver et al., 1995; Korenke et al., 1995; Geel et al., 1997; Moser, 1997). The most common clinical forms are ALD and AMN. ALD manifests clinically before 10 years of age and involves a rapid progression of neurologic symptoms, leading to a vegetative state of the patient, often within 3 years (Moser et al., 1991, 1994; Scriver et al., 1995; Korenke et al., 1995; Moser, 1997). AMN appears between 20 and 40 years of age and is characterized by progressive paraparesis involving disorders of the spinal cord (Moser et al., 1991; Korenke et al., 1995; Geel et al., 1997; Moser, 1997). Ap-
poor diet, normalized plasma C26:0 levels within one month.

The first treatment of X-ALD consisted of restricted ingestion of VLCFA. However, this diet did not succeed since VLCFA are also produced endogenously (Scriver et al., 1995; Moser, 1995, 1997). A diet combining VLCFA restriction and ingestion of monounsaturated oleic acid (glyceroltrioleate, GTO) was proposed and was found to reduce plasma C26:0 levels by 50% within 4 months in X-ALD patients (Scriver et al., 1995; Moser, 1995, 1997). It was later demonstrated that a mixture of monounsaturated erucic acid (glyceroltrierucate, GTE) with GTO at a 4:1 proportion, called Lorenzo’s Oil, combined with a VLCFA-poor diet, normalized plasma C26:0 levels within one month (Scriver et al., 1995; Moser, 1995, 1997). After a few years of study, however, the effectiveness of treatment with Lorenzo’s Oil was questioned, since it did not prevent the progression of preexisting neurologic symptoms (Scriver et al., 1995; Geel et al., 1997; Restuccia et al., 1999).

The effectiveness of this therapy for patients affected with the X-ALD is disputed. To further investigate this controversy, we evaluated the clinical and biochemical course of 7 Brazilian patients treated with Lorenzo’s Oil and a VLCFA-restricted diet.

**MATERIAL AND METHODS**

Patients and controls

We evaluated 7 patients with X-ALD under treatment with a VLCFA-poor diet and Lorenzo’s Oil. The mean age of ALD patients at the time of diagnosis was 10 years and AMN patients were 29 years old. A total of 40 blood collections were performed to determine plasma VLCFA. A group of 30 normal individuals was also studied to determine normal values. The patient and control groups were of comparable age (7 to 38 years). Blood was collected in the presence of heparin, and plasma was separated and stored frozen at -20ºC until the time of analysis. The study was conducted according to the recommendations of the Ethics Committee of the University Hospital of Porto Alegre and all patients gave written informed consent to participate.

Methods

VLCFA were analyzed by the technique of Moser and Moser, 1991. A 25 µl volume of the internal standard (C27:0-heptacosanoic acid) was added to 100 µl of plasma and VLCFA were extracted with a chloroform:ethanol mixture (1:1). After centrifugation, the supernatant was mixed with distilled water and chloroform to remove the precipitated protein. After a new centrifugation, the lower phase was evaporated with nitrogen vapor (N2) and chloroform:methanol (2:1) was used to redissolve the lipids. Methanol-HCl (3 N) was added to each dried total lipid extract and the tubes were thoroughly sealed and kept at 75ºC for 16 h for the formation of fatty acid methyl esters, which were then purified by thin-layer chromatography. Plates (0.25 µm silica gel G 20 x 20) were prewashed with chloroform:methanol:acetic acid:water (52:20:7:3) and hexane:ether:acetic acid (90:10:1). After sample application to the plate, the chromatographic run was performed using toluene:ether (97:3) as eluent. After air drying for 16 h, the samples were stained with iodine vapor. The VLCFA methyl esters were extracted from the silica 3 times using hexane. After drying with N2 vapor, the samples were redissolved with 50 µl hexane and analyzed by gas chromatography using a Varian apparatus equipped with an HP-5 column (5% methylphenyl silicone, 0.33 µm film thickness, 0.2 mm inner diameter and 25 m in length), a flame ionization detector, and an injector split/plitless type, using helium as the mobile phase. The concentrations of C22:0 (docosanoic acid), C24:0 (tetracosanoic acid) and C26:0 (hexacosanoic acid) were determined and C26:0/C22:0 and C24:0/C22:0 ratios were calculated.

**Treatment**

The patients in the study were treated with a VLCFA-restricted diet and 1.2 ml kg⁻¹ day⁻¹ Lorenzo’s Oil (Moser et al., 1992; Geel et al., 1997). Doses were tailored based on platelet numbers and plasma VLCFA levels of the individuals. Table I indicates the duration of treatment and the doses of Lorenzo’s Oil used by the patients with X-ALD.

Supplementation with multivitamins, minerals and an additional source of essential fatty acids (Moser, 1995), linoleic acid and linolenic acid found in saffron oil and fish oil was recommended. In general, restriction of VLCFA ingestion can be achieved by eating the following foods: non-fatty dairy products, lean beef and pork cuts, chicken without skin and low-fat fish, plus fruits and vegetables. The main foods to be avoided are saturated fats such as industrialized fats (butter, cream, milk, ice cream), fats present in meat and meat derivatives, and vegetable oil.

**Table I - Duration of therapy and doses of Lorenzo’s Oil used for X-ALD patients under dietary treatment.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Oil dose (l/month) and ml kg⁻¹ day⁻¹</th>
<th>Duration of therapy (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2 and 1.2</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>2 and 1.2</td>
<td>33</td>
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<tr>
<td>3</td>
<td>1.6 and 1.2</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>2.0 and 1.6</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>3.0 and 2.0</td>
<td>12</td>
</tr>
<tr>
<td>AMN</td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>3 and 1.2</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>3 and 1.2</td>
<td>27</td>
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</tbody>
</table>
RESULTS

Figure 1 (A, B and C) illustrates the plasma concentration of \( \text{C}_{26:0} \) and the \( \text{C}_{26:0}/\text{C}_{22:0} \) and \( \text{C}_{24:0}/\text{C}_{22:0} \) ratios, respectively, during the various phases of patient treatment. Figure 1A shows that \( \text{C}_{26:0} \) levels were markedly decreased in all patients after the beginning of treatment and remained relatively close to normal values throughout most of the treatment period. Figure 1B shows that the \( \text{C}_{26:0}/\text{C}_{22:0} \) ratio was reduced in all patients except patient ALD 2 after the beginning of therapy, although this ratio was reduced in this patient during the third phase of treatment. The ratio was reduced to levels closer to normal in all patients.

A wide range of \( \text{C}_{24:0}/\text{C}_{22:0} \) ratios (Figure 1C) was observed throughout treatment in all X-ALD patients, except patient ALD 3 who presented a more consistently normal \( \text{C}_{24:0}/\text{C}_{22:0} \) ratio.

Table II describes the clinical history of X-ALD patients under dietary treatment. Most patients showed no major complications during therapy. Gastrointestinal disturbances like vomiting and diarrhea, and low platelet count were common findings. Only one patient had a progressively deteriorating clinical picture, and one death occurred during therapy. All ALD patients (and none AMN patient) had abnormal CT scans.

DISCUSSION

We describe here our experience with the treatment of 2 patients with AMN and 5 patients with ALD with Lorenzo’s Oil and diet. The mean duration of treatment was 21 months. All patients were diagnosed late and started treatment when irreversible neurologic symptoms were already present.

The patients presented high plasma \( \text{C}_{26:0} \) levels and \( \text{C}_{26:0}/\text{C}_{22:0} \) ratios at the time of diagnosis.

Plasma \( \text{C}_{26:0} \) levels were above 2.86 µmol/l (normal levels: 0.32 to 1.56 µmol/l) in 62% of the determinations made in the 7 patients treated for X-ALD during the treatment period. However, plasma \( \text{C}_{26:0} \) levels were drastically reduced in these patients after the onset of treatment, remaining, on average, 58% below initial values throughout treatment. In the assessment of treatment, it is important to consider the difficulty in complying with a VLCFA-poor diet and in correctly ingesting the oil, a fact that may lead to variations in plasma VLCFA levels during therapy.

Of these patients 57.1% remained without major complications (ALD patients numbers 2 and 5 and AMN patients numbers 1 and 2, see Table I). During the same period, 28.6% of the patients (ALD patients numbers 3 and

Figure 1 - A, Plasma \( \text{C}_{26:0} \) levels; B, \( \text{C}_{26:0}/\text{C}_{22:0} \) ratio, and C, \( \text{C}_{24:0}/\text{C}_{22:0} \) ratio of X-ALD patients treated with Lorenzo’s Oil at different times. The broken lines indicate the normal ranges. The continuous lines with different symbols indicate the 5 ALD and 2 AMN patients.
is unlikely that an effect occurs with the currently prescribed dose of Lorenzo’s Oil. Other analyses have indicated that C_{26:0} levels observed *post mortem* in cerebral tissue of X-ALD patients treated with Lorenzo’s Oil do not differ from those of untreated patients (Moser, 1995).

Recent studies have demonstrated that not only C_{24:0} and C_{26:0} are elevated in X-ALD, but also the monounsaturated VLCFA C_{24:1} (Sandhir et al., 1998). In the present study, wide variability in the C_{24:0}/C_{22:0} ratio was observed in most of the patients, probably due to the lack of administration of the essential fatty acids linolenic acid and linoleic acid. Taken together, it seems that treatment based on a special diet combined with Lorenzo’s Oil should be reevaluated.

We conclude that new therapies are needed for X-ALD in order to obtain a better prognosis for the patients and to slow or reverse the progression of symptoms. Other therapies such as bone marrow transplantation or immunosuppression have been used for X-ALD treatment but in most cases without satisfactory results (Scriver et al., 1995; Geel et al., 1997; Moser, 1997). In addition, new drugs such as lovastatin and sodium phenylacetate, which increase peroxisomal β-oxidation of VLCFA, are being tested in order to normalize tissue VLCFA levels, and appear to provide a more effective clinical response (Singh et al., 1998). Gene therapy also holds promise as a potential future treatment of X-ALD.

**RESUMO**

A adrenoleucodistrofia ligada ao X (X-ALD) é uma desordem hereditária do metabolismo peroxissomal, bioquimicamente caracterizada pelo acúmulo de ácidos graxos de cadeia muito longa (‘very long chain fatty acids’- VLCFA) em diferentes tecidos e em fluidos biológicos, sendo os principais ácidos acumulados o hexacosanoico (C_{26:0}) e o tetracosanoico (C_{24:0}). O acúmulo destes ácidos graxos está associado com desmielinização cerebral e insuficiência adrenal. A incidência desta condição é estimada em 1 para 25.000 em homens. Pelo menos seis fenótipos podem ser diferenciados, sendo a adrenoleucodistrofia (ALD) cerebral infantil e a adrenomieloneuropatia (AMN) os mais comuns. O tratamento preconizado consiste na utilização da mistura gliceroltrioleato/gliceroltrierucato (GTO/GTE), conhecida como Óleo de Lorenzo, combinada com dieta pobre em VLCFA. Existem ainda, terapias alternativas como transplante de medula óssea e imunossupressão, além da utilização de lovastatina e fenilacetato de sódio. Neste trabalho fez-se uma avaliação do tratamento com Óleo de Lorenzo associado à dieta restrita em VLCFA de 7 pacientes homens com X-ALD analisando a evolução clínica e bioquímica. Os pacientes apresentaram uma redução média de 50% nos valores de C_{26:0} e de 42,8% na razão C_{26:0}/C_{22:0} após o início do tratamento. A maioria dos pacientes permaneceu clinicamente bem e aproximadamente 30% dos pacientes apresentaram uma progressão rápida no curso clínico da doença. Parece não haver uma clara correlação bioquímico-clínica do tratamento. Os resultados nos mostram que novas terapias mais eficazes para X-ALD são necessárias para que se possa obter um melhor prognóstico da doença com progressão mais lenta dos sintomas apresentados ou mesmo reversão dos sintomas já presentes nos pacientes.
REFERENCES


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