Annu. Rev. Nutr. 1995. 15:379–97

DIETARY MANAGEMENT OF X-LINKED ADRENOLEUKODYSTROPHY

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KEY WORDS: very-long-chain fatty acids, erucic acid, dietary therapy, myelin, adrenal insufficiency

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0199-9885/95/0715-0379$05.00

ABSTRACT

Adrenoleukodystrophy (ALD) is an X-linked disorder that involves mainly the nervous system white matter and adrenal cortex. It is associated with the accumulation of saturated very-long-chain fatty acids (VLCFAs), such as hexacosanoic acid (C26:0), that occurs as a result of the impaired capacity to degrade these substances, a reaction that normally takes place in the peroxisome. The VLCFAs originate from the diet and are also synthesized endogenously. Interest in dietary therapy arose from the observation that the administration of oils containing erucic and oleic acid (Lorenzo's oil), when combined with restriction of dietary intake of VLCFAs, can normalize plasma VLCFA levels in ALD patients. Clinical results in patients who are already symptomatic have been disappointing. However, preliminary data, still in need of confirmation, suggest that dietary therapy begun in asymptomatic patients can reduce the frequency and severity of later neurological disability.

INTRODUCTION

The term adrenoleukodystrophy (ALD) is applied to two entirely distinct entities: X-linked ALD and neonatal ALD. This review is confined to X-linked ALD. Neonatal ALD (20) is an autosomal recessive disorder related to the Zellweger cerebrohepatorenal syndrome (74), in which the peroxisome fails to form normally (27). Neonatal ALD was named before the nature of the disease process was understood. Its management differs fundamentally from that of X-linked ALD (27) and is not reviewed here. In the subsequent discussion, the abbreviation ALD refers exclusively to the X-linked form of the disease.

Although dietary management of ALD has now been conducted for more than 4 years and has involved more than 400 patients, its clinical effectiveness is still uncertain owing to the great variability of the clinical course of ALD in untreated patients and to the fact that ethical considerations prohibit the use of a randomized, placebo-controlled study design.

X-LINKED ADRENOLEUKODYSTROPHY

Clinical Features

ALD presents with a variety of phenotypes. The childhood cerebral form (62) affected 48% of patients in a series of 1475 ALD patients tested at the Kennedy

Krieger Institute. The mean age at onset of symptoms was 7.1 ± 1.7 years (42). Hyperactivity and diminishing school performance are the most common initial symptoms, and the patient may be misdiagnosed as having an attention deficit disorder. Indicators of a more serious underlying disorder include rapid progression of behavioral disturbances, signs of dementia, seizure, and visual, auditory, or motor deficits. The illness often progresses rapidly and may result in an apparently vegetative state within 2 years and death at various intervals thereafter.

Of the 1475 patients in this series, 25% had the adrenomyeloneuropathy (AMN) phenotype. This phenotype presents in early adulthood (mean age at onset is 27.6 ± 8.7 years) with a progressive paraparesis and sphincter disturbances resulting from involvement of the long tracts in the spinal cord. AMN progresses slowly over several decades. Many patients have been gainfully employed and have raised families, and some have survived to their seventies. Approximately half of all AMN patients may also develop cerebral involvement during the course of the illness; under these circumstances, the disease may progress rapidly. AMN is often misdiagnosed as multiple sclerosis.

More rarely, cerebral symptoms begin in adolescence (5%) or adulthood (3%) and then progress as rapidly as they do in the childhood cerebral form, although intermediate rates of progression are also observed. Approximately 10% of ALD patients have the Addison only phenotype, i.e. adrenocortical insufficiency without apparent neurological involvement, although many of these patients develop AMN in their late twenties or later. Of the 1475 patients in the Kennedy Krieger series, 8% were asymptomatic. Half of these were young children still at risk of developing the childhood cerebral form of the disease, whereas the other half were adolescents or adults. Most of these patients will later develop AMN. We know of only two patients >50 years of age who were reported to be free of neurological or adrenal involvement (37). We believe that the above figures overestimate the proportion of severely involved patients, since the diagnosis is missed more often in the mildly involved patient. Nearly all male patients with ALD have varying degrees of adrenal insufficiency that must be searched for and treated (35).

Approximately 20% of women who are heterozygous for ALD develop neurological disability that resembles that seen in AMN but that is milder and of later onset (40). Less than 1% of heterozygous women have adrenal insufficiency.

Morphological Changes

The pathology of ALD was reviewed recently (49, 50). Postmortem studies of the brain in childhood show that the cortex is usually intact but that the centrum semiovale is replaced by large areas of gray-to-brown firm translucent tissue. This confluent and often bilaterally symmetric demyelination is most com-

monly observed in the parietooccipital region. Diffuse infiltrations and perivascular collections of mononuclear cells, mostly lymphocytes, are seen frequently. In AMN, the spinal cord is involved most severely. The pattern of fiber loss is consistent with a distal axonopathy. The greatest fiber losses are observed in the lumbar corticospinal, cervical gracile, and dorsal spinocerebellar tracts. Perivascular accumulations of lymphocytes or macrophages are milder than in the childhood cerebral form and may be absent. Peripheral nerve involvement is less severe than in the CNS. Sural and peroneal nerves may exhibit a loss of fibers both large and small in diameter. In the adrenal cortex, cells become ballooned first owing to abnormal accumulation of lipids in the inner fasciculate and reticular zones. As the disease advances, the cortex becomes severely atrophic (51). Postpubertal males exhibit lipid inclusions in the Leydig cells similar to those in the adrenal cortex. Ultrastructural studies show dense bileaflet structures lying free in the cytoplasm of adrenocortical, Schwann, and Leydig cells and in macrophages in CNS white matter lesions (51–54). These inclusions are characteristic of ALD.

Biochemical Abnormalities

The principal biochemical abnormality in ALD is the accumulation of unbranched saturated very-long-chain fatty acids (VLCFAs), particularly hexacosanoic acid (C26:0) and tetracosanoic acid (C24:0). Pentacosanoic acid (C25:0) and lesser amounts of fatty acids with carbon chain lengths of 30 or more are also present. Some degree of excess of these saturated VLCFAs is noted in all lipids and tissues, but the greatest excess occurs in the adrenal cortex and brain white matter (18), particularly in the cholesterol ester fractions in these tissues, where saturated VLCFAs may comprise 20–67% of total fatty acids compared with 0–5% in controls. Abnormally high VLCFA levels also are present in brain gangliosides (17). Additionally, an excess of VLCFAs is seen in myelin lipids such as sulfatides or cerebrosides, although in these lipids, which normally contain VLCFAs, the relative increase over normal is of the order of 1.5–2 (9), less than that observed in the cholesterol ester or ganglioside fractions. In brain white matter, the pattern of VLCFA excess in various lipid classes varies with the degree of histopathology. The VLCFA excess in the cholesterol ester fraction is most striking in those regions undergoing active demyelination (56, 67). In brain regions where myelin is still histologically intact, the cholesterol ester fraction has a normal fatty acid composition, and the VLCFA excess is greatest in the phosphatidylcholine fraction (67).

Abnormalities of VLCFA Metabolism in ALD

In 1984, Singh et al showed that patients with ALD had an impaired capacity to degrade VLCFAs (64). This degradation normally takes place in the peroxisome (63). Production of [^{14}C] CO_2 from [^{14}C] C24:0 or from [^{14}C] C26:0

was reduced to 17% of control in cultured skin fibroblasts, leukocytes, and amniocytes of ALD patients, whereas no abnormality was apparent in the oxidation of palmitic (C16:0) or stearic (C18:0) acids. Lazo et al (28) and Wanders et al (73) determined that the defect resulted from an impaired capacity to form the coenzyme A derivative of C24:0, a reaction catalyzed by VLCFA CoA synthase.

Although investigators generally agree that the VLCFA excess in ALD results from an impaired capacity to degrade these substances, Tsuji et al demonstrated a 50–80% increase over control in the microsomal fatty acid elongating system in cultured skin fibroblasts of ALD patients (68). Thus, increased endogenous synthesis also may contribute to VLCFA accumulation in ALD.

Genetics of ALD

The incidence of ALD is unknown. The Peroxisomal Disease Laboratory at the Kennedy Krieger Institute diagnoses approximately 80 new patients annually. This figure would give an incidence of 1.1:100,000 based on 3.6 million births per year in the United States, but this is clearly a minimum value because not all samples for ALD testing are sent to this laboratory, and not all at-risk patients are tested. Mosser et al estimated the incidence to be 1 in 20,000 (45). ALD has been diagnosed in all races worldwide and does not appear to favor any particular race or region (37). The pattern of inheritance has been X-linked recessive in all of the more than 300 pedigrees for which information is available (37).

As noted above, the severe childhood cerebral and the milder AMN phenotype frequently occur together in the same kindred or even nuclear family. Segregation analysis indicates a 20:1 likelihood that this intrafamilial phenotypic heterogeneity is due to the action of an autosomal modifier gene (42). This finding is discussed in more detail in the section on the pathogenesis of ALD.

The ALD Gene

Although the ALD gene was mapped to Xq28 in 1981 (30), it was not isolated until 1993 (45). The gene, which is comprised of 10 exons, spans ~21 kb of genomic sequence and encodes a predicted mRNA with an open reading frame of 2625 base pairs encoding a protein of 834 amino acids (61). It has a high degree of sequence similarity with the peroxisomal membrane protein, PMP70 (45), which is a member of a superfamily of transmembrane transporters, the ATP-binding cassette proteins (16). The ALD protein (ALDP) is a peroxisomal integral membrane protein (12) that may be required in some as yet unidentified manner for the transport or binding of the VLCFA coenzyme synthase, or of VLCFAs or their CoA derivatives.

A variety of mutations of this gene have been identified in patients with ALD (13, 21). We have demonstrated mutations in 24 of 25 unrelated ALD patients (24). Except for one identical AG deletion in exon 5, which we found to be present in 12% of patients (24), the mutations differed among families, and there was no correlation between the nature of the mutation and the phenotype.

Diagnosis

The diagnosis of ALD is based on clinical evaluation and on the demonstration of elevated levels of saturated VLCFAs in plasma (38), red blood cell membranes (69), leukocytes (31), cultured skin fibroblasts (39), or cultured amniocytes (41). These assays are reliable indicators of the disease in affected males. Plasma levels of VLCFAs are already elevated at birth (AB Moser, unpublished observations). Most heterozygous women also have elevated levels of VLCFAs in plasma or cultured skin fibroblasts (43), but false negative or equivocal results are obtained in approximately 15% of women known to be obligate heterozygotes. Linkage analysis with the DXS52 probe (4) has been employed to improve the accuracy of heterozygote identification, but crossovers between this probe and ALD have been observed (KD Smith, unpublished observations). Definitive identification of heterozygote status can be achieved when the mutation has been defined in a male proband. However, because the mutations vary among families and in some instances have not been demonstrable, this procedure cannot yet be offered as a clinical service. Brain magnetic resonance (26) or spectroscopy (25) studies often show rather characteristic changes in patients with the cerebral forms of ALD, but these studies often are normal in patients with AMN or with the Addison only or asymptomatic phenotype.

PATHOGENESIS OF ALD

Normal Distribution and Sources of Saturated VLCFAs

The highest concentration of saturated VLCFAs is found in myelin lipids and red blood cell sphingomyelin. C26:0 accounts for 1–5% of total fatty acids in brain cerebrosides and sulfatides (9, 18) and in red blood cell sphingomyelin (69). It constitutes ~0.01% of total fatty acids in plasma (38) and adipose tissue (55) and ~0.02% of total fatty acids in the normal adrenal gland (41).

Synthesis of VLCFAs

Synthesis of fatty acids with chain lengths >16 carbons is carried out by a fatty acid elongation system. This system is found in both mitochondria and mi-

crosomes. The microsomal system appears to be more active and to have greater physiological significance than the mitochondrial system (46). VLCFA synthesis is achieved by repeated additions of malonyl CoA so that two carbon units are added until the desired chain length is achieved. Fatty acid synthesis and elongation are complex and highly coordinated processes involving the coordinated action of multiple enzymes and acyl carrier proteins (72). Formation of saturated VLCFAs, including C26:0, from [1-^{14}C] C18:0 was first demonstrated in rat sciatic nerve (11) and subsequently in normal and ALD cultured human skin fibroblasts (68). Bourre et al concluded that a single enzyme is responsible for the elongation of behenic acid (C22:0) and its monounsaturated counterpart, erucic acid (C22:1) (7). This finding is the theoretical basis for the dietary therapy of ALD.

Sources of Saturated VLCFA Excess in ALD

The saturated VLCFAs that accumulate in ALD appear to be derived both from the diet and from endogenous synthesis. The contribution of dietary lipids was demonstrated in a study by Kishimoto et al (22) of a terminally ill patient with ALD being fed by nasogastric tube. The patient was given 10 mg/[3,3,5,5-$^{2}H_4$] C26:0 per day, a quantity equivalent to that found in the usual American diet (71), for the last 100 days of his life. Postmortem study showed that in certain parts of the brain, up to 90% of C26:0 contained the label, indicating that this quantity of C26:0 had been derived from what the patient had eaten during the last 100 days of his life. This result led to the development of a diet that restricts the intake of saturated VLCFAs (71).

Despite this striking result, subsequent studies suggested that the bulk of C26:0 that accumulates in ALD is derived from endogenous synthesis. This conclusion is based on the aforementioned demonstration that active fatty acid elongating systems are present in nervous tissue (11) and cultured skin fibroblasts (68) and on the observation that these systems appear to be more active in ALD cells than in control cells. When another terminally ill patient with ALD was given 50 ml D^2O during the last 196 days of his life, the deuterium enrichment of C24:0 and C26:0 was 72–79% of that in C16:0 and C18:0 acids (44). In contrast, in a study in a patient with Refsum's disease (66) to whom D_2O was administered in a similar fashion, no significant deuterium enrichment of phytanic acid was observed. These studies highlight the difference between ALD and Refsum's disease. In Refsum's disease, the "offending" metabolite is of dietary origin exclusively, and dietary restriction of phytanic acid reduces the plasma and tissue levels of this substance and has a highly favorable effect on the clinical course of this disorder (65). In ALD, however, dietary restriction of VLCFAs failed to reduce plasma levels or alter the clinical course (10).

Effect of VLCFA Excess on Lipid Membranes

Knazek et al (23) demonstrated that red cell membrane microviscosity is increased in patients with ALD. Whitcomb et al (75) studied the effects of abnormally high C26:0 on the function of cultured adrenal cells. They added C26:0 in levels comparable to those found in ALD plasma to the medium of an adrenal cell culture and demonstrated that the microviscosity of the cultured cells was increased. Additionally, they reported that these cells had an impaired response to adrenocorticotrophic hormone (ACTH). The authors concluded that an excess of C26:0 alters membrane function and may thus affect the pathogenesis of ALD. This concept is reinforced by the very recent observations of Ho & Hamilton. These authors conducted studies with [^{13}C] C26:0 enriched in the carboxyl carbon. In mixtures of model membranes and bovine serum albumin, the C26:0 partitioned mainly to the phospholipid bilayers. Under the same conditions, shorter-chain fatty acids partitioned mainly to albumin. The rate of transfer of C26:0 from the bilayers to albumin was four orders of magnitude slower than that of the shorter-chain fatty acids, and bovine serum albumin bound only 1 mole C26:0. In contrast, oleic acid (C18:1) and C16:0 had three high-affinity binding sites and several low-affinity binding sites (J Ho & JA Hamilton, unpublished observations). Membrane models suggest that the excess of C26:0 has a disruptive effect on membrane structure. It seems plausible that abnormally high levels of C26:0 and other saturated VLCFAs impair the structure and function of neuronal membranes in a manner not yet specified. We hypothesize that alterations in membrane structure play a role in the pathogenesis of the axonopathy and tract degeneration associated with the slowly progressive forms of ALD, such as AMN.

The Pathogenetic Role of the Brain Inflammatory Response

As discussed above, approximately half of all patients with ALD experience rapidly progressive demyelination in the cerebral hemispheres. This process is associated with perivascular lymphocytic infiltrates that resemble those seen in multiple sclerosis. This inflammatory response appears to be the hallmark of the rapidly progressive forms of ALD. Griffin et al (14) typed these cells and found that they consisted of 34% T4 cells, 16% T8 cells, 24% B cells, and 11% monocytes and macrophages, a pattern resembling that found in the CNS during a cellular immune response. Powers et al (50) demonstrated high levels of tumor necrosis factor–α in macrophages and, surprisingly, in astrocytes at the advancing edge of the demyelinative lesions. They proposed that the VLCFA excess stimulates nearby astrocytes, perivascular cells, and macrophages to initiate a cytokine-mediated cascade that leads to the superimposed destruction of myelin by T cells and, to a lesser extent, by complement and B cells. Approximately half of ALD patients do not experience this inflammatory

response or its associated rapid neurological progression. We hypothesize that the postulated autosomal modifier gene (42) acts by modulating the severity of the inflammatory response.

DIETARY MANAGEMENT OF ALD

Design and Therapeutic Trial of a VLCFA-Restricted Diet

The initial impetus for dietary therapy was the observation by Kishimoto et al (22) that the C26:0 that accumulated in the brain of an ALD patient was at least partially of dietary origin. This finding led to the development of a diet that restricted the daily intake of C26:0 to ≤3 mg compared with the estimated values of 12–40 mg contained in the customary American diet (71). Because the existing dietary tables did not provide information about the levels of C26:0 or longer-chain fatty acids in foodstuffs, design of this diet required the analysis of VLCFAs in 135 common foods. Subsequently, a cookbook was developed and published by the United Leukodystrophy Foundation (6). This 274-page cookbook includes many recipes developed by families of ALD patients and has been of practical help to many families.

Results of a clinical trial with this diet were reported in 1982 (10). Seven patients with ALD were placed on the diet for periods varying from 4 months to 2 years. Results were disappointing. VLCFA levels in plasma were not reduced, and the neurological disability continued to progress. The oral administration of carnitine or clofibrate also had no demonstrable effect.

Development and Therapeutic Trial of a Glyceryl Trioleate Regimen

In 1986 Rizzo et al (60) reported that the addition of oleic acid to the medium in which cultured skin fibroblasts of ALD patients were growing resulted in a striking reduction in the levels of C26:0. They concluded that the monounsaturated fatty acids competed with saturated fatty acids for the microsomal fatty acid elongating system, which, as shown by Bourre et al (7), uses both types of fatty acids as substrates. This finding led to the design of a new dietary regimen, which combined the administration of glyceryl trioleate (GTO) oil with dietary restriction of VLCFA intake (33, 59). Depending on weight and caloric requirements, patients received 45–90 ml/GTO per day. This dosage provided ~25% of total calories. The fats in the VLCFA-restricted diet provided 10–15% of total calories, so that the total fat intake approximated the 35–40% of calories consumed in the usual American diet. Patients either took the GTO oil separately or incorporated it into foods during cooking, e.g. by using recipes in the ALD-AMN cookbook mentioned above (6). A multivitamin and mineral supplement was prescribed for all patients. Most patients

were able to adhere to the diet. Twenty-eight percent complained of constipation, which was eased by adding foods high in fiber. Ten percent experienced nausea after taking the oil, which was relieved by taking the oil as an emulsion. Of the 20 adult patients, 6 lost weight, but 4 of these had sought to do so.

Unlike the VLCFA-restricted diet alone, the GTO regimen resulted in a lowering of plasma C26:0 levels. On average, these levels fell 50% after 4 months. In several of the heterozygous women, plasma C26:0 levels normalized.

The clinical effects of the GTO regimen were assessed in a 1-year prospective study that involved 34 men with AMN and 16 heterozygous women with AMN-like neurological disability (34). Twenty-five pairs matched for degree of neurological disability, sex, and age were formed. One member of each pair was placed on the GTO regimen, whereas the other member continued his or her customary diet. Neurological status, brain magnetic resonance studies, cognitive function, and neurophysiological functions were evaluated at baseline, 6 months, and 1 year. Nutritional follow-up and VLCFA measurements were performed monthly. As expected, the number of VLCFAs in plasma remained unchanged in the control group, whereas the GTO group experienced a 50% drop in plasma VLCFAs on average.

During this relatively short period of observation, the neurological and functional status did not change substantially in either group. However, analysis of peripheral nerve function did show differences. For the purpose of this analysis, patients were divided into two groups. The first group contained 21 patients whose mean plasma levels of C26:0 during the year of the trial were reduced to <70% of baseline, whereas the second group comprised 29 patients whose mean plasma levels during the year of the trial were >70% of baseline. In the first group, 7 of 12 attributes of peripheral nerve function improved during the year of therapy. For two of the parameters, namely sural and peroneal nerve amplitude, the changes achieved statistical significance. In the first group, these functions improved, whereas in the second group they did not. p values for the differences in the sural and peroneal nerve values were 0.04 and 0.009, respectively. These findings, although not clinically significant in terms of function or muscle strength, were encouraging. Peripheral nerve is capable of regeneration, and the results suggested that reduction of VLCFA levels can have some positive effect, albeit a slight one, on neurological function. Neither we (33) nor Rizzo et al (59) conducted a randomized trial of the GTO oil for patients with the rapidly progressive cerebral forms of ALD. Both groups had the impression that in these GTO-treated patients, the disease process continued to progress at an unaltered rate.

Erucic Acid

Erucic acid (*cis*-13-docosenoic acid) is present in vegetable oils from rapeseed, mustard seed, and crambe seed, all of which are members of the *Cruciferae*

family (15). Rapeseed oil containing 49% erucic acid produces a transient cardiac lipidosis in rats and pigs but not in monkeys or humans (8, 29). As a result of these studies, the use of rapeseed oil high in erucic acid is not recommended by the World Health Organization and is prohibited in many countries.

Development of Lorenzo's Oil

In light of the concern about the safety of oils high in erucic acid and the possibility that patients with ALD might not be able to metabolize this very-long-chain monounsaturated fatty acid, the medical community, ourselves included, was hesitant to consider the use of erucic acid for ALD therapy. However, as depicted so vividly in the motion picture *Lorenzo's Oil,* the Odone family, whose son Lorenzo is severely disabled by ALD, persuaded the Croda Universal Ltd. Company in England to produce a highly purified oil comprised of a 4:1 mixture of glyceryl trioleate and glyceryl trierucate. This mixture is now referred to as Lorenzo's oil in honor of their son. In collaboration with Dr. William Rizzo and associates (57), the Odones demonstrated that this oil has a powerful effect on the levels of saturated VLCFA assays in plasma. The oil normalized the plasma level of C26:0 within 4 weeks and appeared to have few side effects.

Decision to Conduct an Open Rather than a Randomized Trial of Lorenzo's Oil

The dramatic biochemical effect of Lorenzo's oil led us and others to abandon the trials with GTO oil alone and to undertake clinical evaluations of this new substance. The underlying hope was that, as in phenylketonuria or Refsum's disease, the elimination of the excess of the abnormal metabolite (C26:0) might be of dramatic clinical benefit, particularly in the early stages of the disease. This hope was heightened by the aformentioned observation that the moderate reduction of C26:0 levels achievable with the GTO oil did appear to improve peripheral nerve function (34). When we balanced the frightening prognosis of untreated ALD against this highly encouraging biochemical effect and the apparent safety of the dietary therapy, we concluded that it would be ethically unacceptable to propose a randomized prospective study. This decision was endorsed by the Institutional Review Board at the Johns Hopkins Medical Institutions as well as by the National Institutes of Health and the Food and Drug Administration, which awarded an Investigational New Drug (IND) application and research support from the office of Orphan Drug Products Development.

The Lorenzo's oil dietary regimen represents only a slight modification of the GTO diet described above. Patients continue to follow a diet low in saturated VLCFAs (71), with ~10% of calories derived from fat in foods.

Lorenzo's oil is administered in a dosage that provides ~20% of total caloric intake, which is often accomplished with a daily intake of 2–3 ml/kg body weight. Safflower oil is added in an amount that ensures that 5% of total calories are derived from linoleic acid. Fish oil capsules, which contain negligible amounts of C26:0, are added in amounts that provide ~240 mg docosahexaenoic acid (C22:6) and ~360 mg eicosapentaenoic acid (C22:5) daily. Multivitamin and mineral supplements are given in an appropriate amount based on the patient's age. Detailed nutritional advice and supervision are provided. The majority of patients have been able to adhere to the diet despite its complexity.

Effect of Lorenzo's Oil on VLCFA Levels in Plasma and Brain

The majority of patients following the above regimen achieve normalization of C26:0 levels in plasma and in red blood cells (2, 42, 57, 58). The microviscosity of red blood cell membranes also normalizes (34), presumably as a consequence of the normalization of C26:0 levels in red blood cells. In contrast, C26:0 levels in postmortem brain tissues of four ALD patients who had been treated with Lorenzo's oil for periods of 3 to 16 months did not differ from those in untreated patients (48, 55). The tissues of these patients were also analyzed for erucic acid, the active component of Lorenzo's oil. Although the liver and adipose tissues contained substantial amounts of erucic acid, the levels of this substance in brain were similar to those in untreated ALD patients and in normal controls. This finding indicates that Lorenzo's oil does not penetrate the brain, at least not in substantial amounts. Because the main mode of action of Lorenzo's oil is to diminish the rate of endogenous synthesis of VLCFAs, and because VLCFAs are synthesized in brain (7), these results weaken the theoretical basis of Lorenzo's oil therapy. However, in an ALD patient who had been treated with Lorenzo's oil for a longer period (35 months) than the other patients, C26:0 levels in total lipids, glycolipids, and phospholipids in the brain had normalized, even though they were still abnormally high in the cholesterol ester fraction of the brain (55). Despite this apparent reduction in brain C26:0 levels in this patient, the level of erucic acid in the brain was not increased. In this patient, the prolonged lowering of plasma C26:0 levels may have led to a reduction of levels in the brain, even though Lorenzo's oil failed to enter the brain.

Side Effects of Lorenzo's Oil

The most frequent side effect of Lorenzo's oil is a reduced platelet count, which occurs in ~40% of patients (2, 76, 77). Platelet levels may fall to 100,000/mm^3 and occasionally to 50–80,000 mm^3. The platelet count is inversely proportional to the level of erucic acid in platelet lipids (TS Kickler, unpublished observations) and increases again 2–6 weeks after administration

of Lorenzo's oil is discontinued. No significant abnormal bleeding has been observed. Administration of GTO oil does not affect platelet count. We monitor platelet counts on a monthly or bimonthly basis and replace Lorenzo's oil with GTO oil when the levels fall below 80,000/mm^3. Once the count has increased, Lorenzo's oil therapy is resumed at a lower level, with continued monitoring.

Another side effect of Lorenzo's oil is a reduction in plasma and red blood cell levels of polyunsaturated fatty acids such as C22:6 or arachidonic acid (C20:4) (AB Moser, unpublished observations). The mechanism of this reduction has not been defined, and it can be corrected at least in part by the administration of safflower oil and fish oil capsules. Because these polyunsaturated fatty acids may play an important role in retinal function and brain development (5), this side effect of Lorenzo's oil therapy has led us to postpone its initiation until after the age of two. We have not observed cardiac dysfunction or evidence of cardiac lipidosis in patients receiving this therapy.

Clinical Effects of Lorenzo's Oil Therapy in Patients with Preexisting Neurological or Adrenal Dysfunction

In general, Lorenzo's oil therapy does not arrest the rapid progression of neurological disability in children or adults with the cerebral forms of ALD (42, 57, 58, 70). We recently conducted a questionnaire-based comparison of the rate of progression in 32 untreated and 42 Lorenzo's oil–treated boys who already were in the rapidly progressive childhood cerebral phase of the illness when therapy was started. Preliminary analysis of the data suggests that in the treated group, mortality and the occurrence of milestones such as the inability to talk or stand were delayed slightly, and for certain aspects of the disorder, the difference between the two groups reached statistical significance. However, the results were of very limited clinical significance, since the disability continued to advance in all patients (HW Moser, unpublished observations).

The available data suggest that Lorenzo's oil therapy does not alter the rate of progression of AMN. Aubourg et al reported the results of a 2-year study of 14 AMN patients who had been treated with Lorenzo's oil. None of the patients exhibited improvement, and functional deterioration was noted in nine (2). Kaplan et al (19) reported on the rate of progression of visual evoked response abnormalities in 70 AMN patients who had been treated with Lorenzo's oil for 1 year. In 23% of patients, the response became more abnormal. Improvement was noted in none. Pearson's R correlation analysis was used to determine if the degree of normalization of plasma C26:0 levels was associated with the deterioration of the visual evoked response. No correlation was found, i.e. normalization of plasma C26:0 levels did not protect against deterioration of the visual evoked response. Still in progress in our clinic is a comparison of the rate of disease progression in 100 AMN patients

treated with Lorenzo's oil with that observed in those patients in the previously reported controlled study of GTO therapy (34). Preliminary analysis of the data does not indicate an amelioration of clinical progression.

Assies et al (1) studied the adrenal and testicular function in five men with AMN who had been treated with Lorenzo's oil for 1 year. Preexisting abnormalities in adrenal and testicular function did not improve, and follicle-stimulating hormone (FSH) levels in plasma increased during the therapy. These authors concluded that the diet did not improve endocrine function.

Clinical Effects of Lorenzo's Oil Therapy in Neurologically Asymptomatic ALD Patients

Although the above results indicate that Lorenzo's oil therapy does not result in significant improvement when it is begun in patients with preexisting neurological disability, preliminary observations indicate that it may reduce the frequency and severity of subsequent neurological disability in asymptomatic patients. This observation is of practical significance, since the neurological manifestations of ALD do not begin until age 3 or later (42), but the metabolic defect can be diagnosed at birth. We have identified more than 80 neurologically asymptomatic patients with ALD and continue to identify additional patients nearly every week.

Evaluation of the efficacy of preventive therapy has been hampered severely by the lack of a historical control group. The capacity to identify asymptomatic patients with ALD was achieved only a few years before the advent of the dietary therapies. Furthermore, the hope generated by the motion picture *Lorenzo's Oil* has created tremendous interest and demand for the initiation of therapy. As a result, nearly all asymptomatic ALD patients are currently on dietary therapy, and we have no information about the prognosis of untreated asymptomatic patients.

We are currently following 53 ALD patients who began dietary therapy while they were free of neurological symptoms (36). The mean age at onset of therapy was 7.5 ± 3.2 years (range 3 to 14 years). Forty of the patients were <10 years old at the beginning of therapy. Mean duration of therapy is 38.8 ± 27 months. Of the 50 patients for whom adequate follow-up is available, 48% have remained entirely well; 24% exhibit slightly abnormal results on magnetic resonance imaging (MRI) but have remained well in respect to neurological examination, cognitive function, and school performance; and 18% have mild to moderate disability. The most important and encouraging finding is that only 10% of patients have died or developed classical childhood cerebral ALD. In contrast, in our previous series, 48% of untreated patients with ALD died or developed the severe childhood cerebral form of ALD before 10 years of age. Our preliminary follow-up thus suggests that, although dietary therapy is not a complete preventive, the course of diet-treated asymptomatic patients is

more favorable than had been expected. However, because of the lack of an adequate control group and the need for follow-up, as well as for other reasons discussed in a recent publication (36), this conclusion is still tentative.

CONCLUDING REMARKS

ALD is a serious disease that is particularly poignant since affected boys develop normally until the age of four or later. Dietary therapy has attracted a great deal of interest because it permits normalization of plasma levels of saturated VLCFAs, the abnormal increase of which represents the principal biochemical abnormality in ALD. Therapeutic trials with a 4:1 mixture of glyceryl trioleate and glyceryl trierucate oils, referred to as Lorenzo's oil, that diminishes the rate of endogenous synthesis of saturated VLCFA and that has been popularized by the motion picture of the same name, have now been conducted for more than 4 years and have involved more than 400 patients. Results of these trials indicate that no significant clinical benefit is obtained when the oil is administered to patients with preexisting neurological disability. This lack of benefit may be due to the fact that the oil does not enter the brain in significant amounts and thus may fail to lower the levels of saturated VLCFAs in that organ. Nevertheless, preliminary results suggest that administration of Lorenzo's oil to neurologically asymptomatic patients, although not an absolute preventive, diminishes the frequency and severity of subsequent neurological disability. Resolution of this question is hampered by the fact that for ethical reasons the trial has been conducted without a prospective control group. This apparently favorable result therefore may be due to ascertainment bias. Several more years of follow-up and study of a larger series of patients are required before this crucial question can be answered definitively. We conclude that although current methods of dietary therapy may be of benefit in some forms of ALD, they clearly do not constitute a cure, and other approaches must be pursued concurrently. These include bone marrow transplantation (3), suppression of the inflammatory response with agents such as beta interferon (47), and gene therapy (32).

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