The X-Linked Adrenoleukodystrophy (X-ALD) and Oxidative Stress
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ABSTRACT. Most of the studies indicate that there is as yet no complete cure for X-ALD. However, methods of the treatment seem to slow rather than treat the disease. One method is the use of Lorenzo’s oil in conjunction with a low fat diet, which may help in cerebral X-ALD. X-ALD is in very close resemblance to another neurodegenerative disease, amyotrophic lateral sclerosis (ALS). One of the believed pathomechanisms of ALS is oxidative stress; therefore, this article’s emphasis on the role of reactive oxygen species in X-ALD. The aim of the present study was to review the literature concerning the advances in the treatment of X-adrenoleukodystrophy (X-ALD, OMIM # 300100) in the last two decades and to shed more light on the link between oxidative stress and X-ALD. This review article may point to a deficit in reactive oxygen species (ROS) scavenging and/or ROS overproduction being involved in the aetiopathology of these neurodegenerative diseases. Consequently, one of the useful neuronal rescue strategies could be the treatment with antioxidant agents. doi:10.1300/J157v06n03_07 [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <docdelivery@haworthpress.com> Website: <http://www.HaworthPress.com> © 2006 by The Haworth Press, Inc. All rights reserved.]

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INTRODUCTION

X-Adrenoleukodystrophy (X-ALD, OMIM # 300100) is a peroxisomal disorder associated with the abnormal accumulation of saturated very long chain fatty acids (VLCFA) in plasma and tissues of patients. X-ALD is an inborn X-chromosome linked disease, therefore it affects males specifically. X-ALD is a recessive inherited disorder that leads to central/peripheral nervous system demyelination, adrenal cortex insufficiency and testis inflammation.\(^1\) It affects boys specifically at a rate of approximately 1 in 20,000.\(^1\)

From a clinical prospective, X-ALD can be divided into four categories. One category involves a cerebral demyelinating form affecting boys between 5 and 12 years (40% of cases). The reasons why demyelination and loss of oligodendrocytes occur in X-ALD are still a mystery. One possibility is that a high concentration of VLCFA in CNS lipids may lead to progressive membrane destabilization and dysfunction in glial cells. In boys carrying a mutated X-ALD gene, myelin might be particularly vulnerable because of its high lipid content and its multilamellar structure. An adult form or adenomyeloneuropathy (AMN, 40% of cases), which affects the spinal cord and peripheral nerves is the second category. A third type also exists where the spinal cords of children older than 10 years are affected. Hence it is called childhood cerebral form (CCER, 10% of cases). Because this damages the adrenal glands, the disorder can begin as Addison’s disease and is also called adrenoleukodystrophy. Finally, 35% of adults with X-ALD develop cerebral demyelination and so called cerebral-AMN.\(^2\) The most devastating type, what Lorenzo Odone has, is cerebral demyelinating X-ALD. When a patient has cerebral demyelinating X-ALD, the nerves in the brain are destroyed. Ten percent of X-X-ALD cases appear as Addison’s disease due to damage of adrenal glands. Children with this disease usually developed AMN by middle age.

Ten years ago, a mutated X-gene in X-ALD patients was identified and mapped to \(ABCD1\) (Xq28), which codes for the peroxisomal ABC half-transporter (75 kDa). Nevertheless, the pathogenesis of the disease is still unclear.\(^3\) \(ABCD1\) gene comprises 10 exons spanning approximately 21 kb of genomic DNA.\(^4\) This genetic defect leads to a reduction in \(\beta\)-oxidation of VLCFAs allowing them to accumulate in abnormal concentrations. Most mammalian fatty acids (>90%) have 16-18 carbon atoms derived from the action of cytosolic fatty acid synthase. This enzyme utilizes acetyl-CoA, malonyl-CoA, and NADPH to elongate fatty acids. Further elongation is carried out in cellular endoplasmic re-
The increase in VLCFA concentrations provide a reliable diagnostic tool for identification of X-ALD.

**Therapeutic Strategies for X-ALD**

Several methods have been reported to slow down the destruction of nerves in patients with X-ALD. These methods include gene therapy, bone marrow transplant, anti-cholesterol drugs and Lorenzo’s oil intake.

The Stop ALD Foundation project along with an international multidisciplinary team concluded that gene therapy applied to a patient’s own stem cells was the best approach to pursue. The main aim of the project is to pursue X-ALD homologue upregulation. This homologue is a way to encourage a gene that exists in its normal form in all X-ALD patients to over-express itself so that it could compensate for the initial X-ALD defect. The Stop ALD Foundation has arranged for GlaxoSmithKline (GSK) to share a small part of their library compounds. A number of experiments have been designed and initiated. Promising results have begun to emerge.

The research also showed that a carrier protein responsible for carrying fat molecules to metabolic sites fails to work properly in patients with X-ALD. Methods for treating cerebral X-ALD are bone marrow transplants and immuno-suppression. The idea is to replace cells that have a defective X-ALD gene with cells that have a normal X-ALD gene. Normal bone marrow cells correctly metabolise VLCFA. The normal donor-derived macrophages that enter the brain might improve cerebral lesion in this case. Macrophages cross the blood-brain barrier and pick up the myelin debris and metabolize their VLCFA. The idea is to replace cells that have a defective X-ALD gene with cells that have a normal gene, and break down fats. When these lipoprotein are taken up by myelinating oligodendrocytes, they can now make myelin with a lesser load of VLCFA.

The Mesenchymal Stem Cell (MSC) therapy project involves researchers from several hospitals in USA and Germany and has also been conducted by the Stop ALD Foundation. This experimental therapy would take MSCs from the bone marrow of adult donors and deliver them into the blood and brains of patients with an advanced stage of X-ALD. In the first phase of MSC trials, these stem cells would be used in conjunction with conventional bone marrow transplants.
As for AMN, no specific treatment has been developed. However, since adrenal disorders like Addison’s disease is present, long-term hormonal replacement may provide treatment.9

HMG-CoA reductase inhibitors such as lovastatin, phosphodiesterase 4 inhibitors such as rolipram, and antitumour agents such as sodium phenylacetate, all have been tested experimentally for X-ALD. The exact mechanisms of action is not clear; however, they normalised VLCFA levels in plasma and skin fibroblasts of X-ALD patients.10 Singh et al. suggested that lovastatin may decrease cytokine synthesis and enhance VLCFA β-oxidation subsequently enhancing myelin repair.11

In the mid-1980s, Augusto and his wife Michaela Odone developed Lorenzo’s oil in Chevy Chase, Maryland, USA after their son Lorenzo exhibited symptoms of cerebral X-ALD. (This story was portrayed in the 1993 film “Lorenzo’s Oil.”) The oil is a mixture of four parts glyceryl trierucate oil and one part glyceryl trioleate oil. When combined with dietary intake of oil, decreased VLCFA levels in plasma by 50% were observed after four months of use.12 Moser et al. showed that treatment of X-ALD with mono-unsaturated fatty acids like oleic acid (C18:1) and erucic acid (C22:1) led to the normalisation of C26:0 levels and suggest a mechanism based on competition between the acid on the elongation machinery in the cell.14 Lorenzo’s oil contains C18 and C22 unsaturated fatty acids; despite the reduction in plasma levels of VLCFA, this oil therapy did not desist neurological progression.15

Role of Free Radicals in X-ALD

The mechanism(s) underlying X-ALD and neuronal damage are poorly known. X-ALD is a demyelinating peroxisomal disorder. The peroxisomal matrix consists of several oxidase enzymes that produce a superoxide anion (O2-) and hydrogen peroxide (H2O2). The matrix also includes enzymes involved in β-oxidation of VLCFA.16,17 Defects in peroxisomal enzyme function are associated with fatal neurological changes during the life span.

It has been shown that lovastatin and sodium phenylate inhibit nitric oxide synthase and the neuroinflammatory process in X-ALD patients.11 Circulating free radicals have been linked to several neurodegenerative diseases.18 Due to the selective vulnerability of neurons, the brain contains additional antioxidant defences. Capillary endothelial cells of cerebral microvessels possess specific and unique features to form the blood-brain barrier (BBB) in order to control the entry of many types of solutes from general circulation to the cerebral parenchyma. It is formed
basically by a monocellular layer of endothelial cells sealed by tight junctions, which possess high levels of enzymatic and non-enzymatic antioxidants. In addition, astrocytes surrounding the BBB contain higher antioxidant concentrations than other brain cell types. Although the BBB has high levels of antioxidant enzymes the brain tissue has only low levels of these defence enzymes.

Bayol-Denizot et al. found that co-cultured astrocytes protect other brain cell types against reactive oxygen species (ROS) whereas cultured neurons are more vulnerable to damage by ROS than astrocytes. In vivo, neurons and astrocytes are in close proximity. Evidence indicates that an intensive metabolic exchange occurs between neuron and astrocyte cells in brain to remove and inactivate neurotransmitter molecules. Such interactions are very important regarding cerebral homeostasis and protection of the brain against xenobiotics and oxidative stress. In addition to the contribution of astrocyte cells in defence systems, it has been reported that they increase the activity of superoxide dismutase, catalase and glutathione peroxidase in BBB endothelial cells. Consequently, astrocytes lower the ROS levels entering the brain and protect against degenerative diseases.

Even at a cellular ratio of one astrocyte cell to 20 neurons, neurons can be damaged by ROS. Compared with all other tissues, the brain is particularly vulnerable to oxidative processes. Neurons of the CNS are almost completely dependent on oxidative phosphorylation reactions in order to generate adenosine triphosphate (ATP) for energy. In addition, normal adult brain depends on glucose as the major nutrient and, therefore, the brain has a high glucose metabolism and respiratory turnover. Thus, high rates of oxygen turnover may account for the vulnerability of CNS neurons to ROS.

The brain contains high concentrations of free iron, which mediates the conversion of hydrogen peroxide to hydroxyl radicals via the Fenton reaction. Furthermore, neuronal membranes of the brain contain high concentrations of polyunsaturated fatty acids, which are potential substrates for peroxidation by hydroxyl radicals. In addition, the loss of neurons in adult brain cannot generally be compensated by neuron regeneration.

Anatomically, motor neurons may be more vulnerable to ROS damage than other neurons. The large cell body and remarkable length of motor neuron axons predict that these cells have high-energy demands and a high metabolic rate, requiring a high level of mitochondrial activity in comparison to other cells. The selective vulnerability of neurons may explain why neurotoxic drugs are able to damage nerve terminals
and why ROS play a significant role in the pathology of several neurological diseases.\textsuperscript{27,28}

X-ALD is a progressive and fatal disease. Neurodegeneration affects primarily motor neurons of the brain and spinal cord. Since X-ALD is a rare disease, there are fewer studies in this area than more prevalent disorders such as atherosclerosis or cancer.

There are several reasons why X-ALD is potentially a useful model for more common neurodegenerative disorders, such as Parkinson’s disease, Alzheimer’s disease, and Huntington’s chorea. First, a common feature of these disorders is selective neuronal death. One can propose that the proximate cause of neuronal death may differ in these diseases, but the final common pathway is likely to be similar.\textsuperscript{29} Secondly, the involvement of the motor system in X-ALD permits simpler and more direct diagnosis than do extrapyramidal changes or dementia in Parkinson’s disease and Alzheimer’s disease, respectively.\textsuperscript{30} Thirdly, the relatively rapid onset (less than two years) and stereotyped natural history facilitate clinical monitoring of the disease.\textsuperscript{31}

A link between free radicals and X-ALD is supported by the selective vulnerability of motor neurons to oxidative stress damage. Recent reports have indicated oxidative changes in proteins, lipids and DNA in the CNS of patients with degenerative diseases.\textsuperscript{32,33} For this reason, antioxidants like recombinant superoxide dismutase and procysteine (a glutathione repleting agent) may merit some activity in X-ALD treatment.

Vargas et al. have demonstrated that an increase of erythrocyte glutathione peroxidase activity and of catalase/superoxide dismutase activities in fibroblasts from patients in comparison to control.\textsuperscript{17} The significant increase in antioxidant enzyme activities is a result of high sustained levels of reactive species in X-ALD patients. The increase in catalase and superoxide dismutase activity suggested the formation of hydrogen peroxide and superoxide anions, respectively. Peroxisomes contain many of the cellular enzymes that generate hydrogen peroxide such as glycolate oxidase, urate oxidase and flavoprotein dehydrogenases involved in β-oxidation of fatty acids.\textsuperscript{16} Recently, Biase et al. have used plasma low-density lipoprotein (LDL) as an indicator for oxidative stress in both X-ALD and AMN patients.\textsuperscript{34} Furthermore, the role of nitric oxide in neurological and demyelinating diseases could be monitored using oxidised LDL.

The most broadly accepted hypothesis for the aetiopathology of Parkinson’s disease is selective oxidative stress in the substantia nigra.\textsuperscript{35} Studies indicate that dopaminergic neurons in Parkinson’s disease may
be more susceptible to oxidative stress due to reduced glutathione levels and excessive free iron content.\textsuperscript{36} Dopamine generates free radicals and hydrogen peroxide by auto-oxidation or through normal enzymatic processing by monoamine oxidase.\textsuperscript{37} Consequently, high levels of hydrogen peroxide are present in the substantia nigra.

It has also been suggested that the neuropathology of Huntington’s chorea involves oxidative stress, although most of the evidence is indirect.\textsuperscript{36} Post-mortem of brains from patients with Huntington’s chorea show an increase in oxidised DNA indicative of oxidative stress damage coupled with reduced levels of superoxide dismutase and oxidised glutathione.\textsuperscript{28}

Evidence for the role of oxidative stress in Alzheimer’s disease aetiology is accumulating.\textsuperscript{38-40} Various products of oxidation reactions like oxidised glutathione molecules, and mediators of oxidative stress, such as accumulation of free fatty acids, are found in brain of patients with Alzheimer’s disease.\textsuperscript{39} Basically, most of the cellular macromolecules (DNA, protein, and lipids) can be found in an oxidised form in Alzheimer’s disease brain tissue.\textsuperscript{39,40} Other studies indicate that superoxide dismutase activity is decreased in the brains of Alzheimer’s disease patients although these results are not substantiated in other studies.\textsuperscript{39-41}

Recently, melatonin has been shown to be highly effective in reducing oxidative damage in Parkinson’s disease, Huntington’s chorea and Alzheimer’s disease. This efficacy derives from its ability to function as a direct and indirect antioxidant.\textsuperscript{42,43} X-ALD may have more than one pathological mechanism which explains the presence of different forms in the same family.

In summary, these findings may point to a deficit in ROS scavenging and/or ROS overproduction being involved in the aetiopathology of these neurodegenerative diseases.\textsuperscript{44} Consequently, one of the useful neuronal rescue strategies could be the treatment with antioxidant agents.\textsuperscript{36,41,45}

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