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The X-linked Adrenoleukodystrophy (Ald) and Oxidative Stress

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Abstract: The objective of this study was To review the literature concerning with the advance in the treatment of X-adrenoleukodystrophy (X-ALD, OMIM # 300100) in the last two decades. To shed more light on the link between oxidative stress and X-ALD. Data for this review article were collected using ERIC-MEDLINE (1986-July 2005), UMI-PQ medical library (1990-December 2004) and science direct (1987-July 2005), using the key words adrenoleukodystrophy, adenomyeloneuropathy, Lorenzo's oil, bone marrow transplant, oxidative stress, antioxidants and neurodegenerative diseases. Search were limited to English and French languages. Articles have been selected from pharmacological point of view where human subject or human samples (serum, urine or tissues) has been used in that particular study. Most of the studies indicate that there is as yet no complete cure for 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 helps in cerebral ALD. ALD is in very close resemblance to another neurodegenerative diseases, amyotrophic lateral sclerosis (ALS). One of the believed pathomechanisms of ALS is oxidative stress, thus this article emphasis on the role of reactive oxygen species in ALD.

Key words: adrenoleukodystrophy, adenomyeloneuropathy, Lorenzo's oil, oxidative stress, antioxidants.

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 affect males specifically. X-ALD is a recessive inherited disorder that lead to central/peripheral nervous system demyelination, the adrenal cortex insufficiency and the testis inflammation[1]. It affect boys specifically with approximately 1/20,000[1].

From clinical prospective, X-ALD divided into four categories: cerebral demyelinating form affecting boys between 5-12 years (40% of cases), adult form or adenomyeloneuropathy (AMN, 40% of cases) which affects spinal cord and peripheral nerves, a third type is also exist when spinal cords of children are affected after 10 years and finally 35% of adult-spinal form may develop cerebral demyelination[2]. The most devastating type, what Lorenzo Odone has, is cerebral demyelinating, meaning that nerves in brain are destroyed. 10% of X-ALD cases appears 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 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 reduction in $-oxidation of VLCFAs allowing them to accumulate in abnormal concentrations. Most of mammalian's fatty acids (>90%) have 16-18 carbon atoms derived from the action of cytosolic fatty acid synthase which utilizes acetyl-CoA, malonyl-CoA and NADPH to elongate fatty acid. More elongation is carried out in endoplasmic reticulum via specific fatty acid chain elongation enzymes which result in formation of VLCFA[5] The increase in VLCFA concentrations provide a reliable diagnostic tool for identification of X-ALD.

Therapeutic strategies for ALD: In literatures several methods have been reported to slow down the destruction of nerves which includes gene therapy, bone marrow transplant, anti-cholesterol drugs and Lorenzo's oil intake.

The stop ALD foundation project along with an international multi-disciplinary team concluded that gene therapy applied to patient's own stem cells was the best

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approach to pursue[6]. The main aim of the project is to pursue ALD homologue up-regulation. This homologue is a way to encourage a gene that exists in its normal form in all ALD patients. The desired effect would be for this normal gene to over-express so that it could compensate for the initial ALD defect. The stop ALD foundation has arranged for GlaxoSmithKline (GSK) to share a small part of their experience and a number of experiments have been designed and promising results have begun to emerge.

The research also showed that a carrier protein fails to work properly and carry the fat molecules to where they would be metabolised. Methods for treating cerebral ALD are bone marrow transplants and immuonsuppression[7]. The idea is to replace cells that have a defective ALD gene with cells that have a normal ALD gene and will break down fats. In fact, normal bone marrow cells can correctly metabolised VLCFA[8]. The normal donor-derived macrophages that enter the brain might improve cerebral lesion in this case[2].

The Mesynchymal Stem Cell (MSC) therapy project involves researchers from several hospitals in USA and Germany has been also conducted by stop ALD foundation. This experimental therapy involves the use of MSCs taken from the bone marrow of adult donors. The MSCs would be delivered into the blood and brains of ALD patients who are at an advance ALD stage. In the first phase trial of MSCs, these stem cells would be used in conjunction with conventional bone marrow transplants[8].

As for AMN, no treatment has yet been developed and because adrenal disorder like Addison's disease is present, long term hormonal replacement could provides treatment[9].

Anti-cholesterol drugs such as lovastatin, phosphodiesterase 4 inhibitor, rolipram, and antitumour agen, sodium phenylacetate, all have been tested experimentally for ALD. The exact mechanism of action is not clear yet. 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 $-oxidation and subsequently enhance myelin repar[11].

In mid of 1980s, Augusto and his wife Michaela Odone developed Lorenzo's oil in Chevy Chase, Maryland, USA after their son Lorenzo exhibit symptoms of cerebral ALD (this story was portrayed in the 1993 film "Lorenzo's oil"). This oil is a mixture of four parts glyceryl trierucate oil and one part glyceryl trioleate oil, which when combined with dietary intake of oil, decrease VLCFA level in plasma by 50% after four month 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 the suggest a mechanism based on competition between the acid on the elongation machinery in the cell[14] Lorenzo's oil contains C18 and C22 fatty acid, despite this parameter this oil therapy did not desist neurological progression(Figure 1)[15]

Role of free radicals in X-ALD: The mechanism(s) underlying X-ALD and neuronal damage are poorly known. ALD is a demyelinating peroxisomal disorder. The peroxisomal matrix consist of several oxidase enzymes that produce superoxide anion ($O_2^{\cdot}$) and hydrogen peroxide (H_2O_2) and enzymes involved in $-oxidation of VLCFA[16,17]. Defects in peroxisomal functions are associated with fatal changes at the neurological level during life span.

It has been shown that lovastatin and sodium phenylate inhibits nitric oxide synthase and the neuroinflammatory process in X-ALD patients[11]. In fact, free radical has been linked to several neurodegenerative disease[18]. Due to the selective vulnerability of neurons, the brain contains additional antioxidant defences. The capillary endothelial cells of cerebral microvessels possess specific and unique features, forming the blood-brain barrier (BBB), which controls 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[19]. In addition, astrocytes, surrounding the BBB, contain higher antioxidant concentrations than other brain cell types[20]. Although the BBB has high levels of antioxidant enzymes; the brain tissue has only low levels of these defence enzymes[21].

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.[22] *In vivo*, neurons and astrocytes are in close proximity. Evidence is indicating that there is an intensive metabolic exchange occurs between neuron and astrocyte cells in brain such as removal and inactivation of neurotransmitter molecules[23] Such interactions are very important regarding cerebral homeostasis and in the protection of the brain against xenobiotics and oxidative stress[20]. In addition to the contribution of astrocyte cells in defence

(a)

(b)

Fig. 1(a): Lorenzo with his old friend Hassane (top) in the Comoros Islands, 1983.
Fig. 1(b): Lorenzo and Hassane nearly 20 years later at home in Virginia, USA, 2001.

systems, it has been reported that they increase the activity of superoxide dismutase, catalase and glutathione peroxidase in BBB endothelial cells[19]. Consequently, astrocytes lower the ROS levels entering the brain and protect against degenerative diseases.

Even at a cellular ratio of one astrocyte cell to twenty neurons, neurons can be damaged by ROS[22]. Compared to all other tissues, the brain is particularly vulnerable to oxidative processes. Neurons of central nervous system (CNS) are almost completely dependent on the oxidative phosphorylation reactions in order to generate adenosine triphosphate (ATP) as energy source[24]. In addition, normal adult brain depends on glucose as the major nutrient and, therefore, the brain has a high glucose metabolism and respiratory turnover[24]. Thus, high rate 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[25]. In addition, the loss of neurons in adult brain cannot generally be compensated by neuron re-generation[24].

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[26].

The selective vulnerability of neurons may explain why neurotoxic drugs are able to damage nerve terminals and the suggested role of ROS in the pathology of several neurological diseases[27,28].

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

However, X-ALD is potentially a useful model for more common neurodegenerative disorders, such as Parkinson's disease, Alzheimer's disease and Huntington's chorea due to several reasons. First, a common feature of these disorders is selective neuronal death. Therefore, 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[29]. Secondly, the involvement of 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[30]. Thirdly, the relatively rapid onset (less than two years) and stereotyped nature history facilitate clinical monitoring of the disease[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[32,33]. For this reason, antioxidants like recombinant superoxide dismutase and procysteine (a glutathione repleting agent) may merit some activity in ALD treatment.

Vargas et al. have demonstrated that an increase of erythrocyte glutathione peroxidase activity and of catalase superoxidase activities in fibroblasts from patients in comparison to control[17]. The significant increase in antioxidant enzyme activities is a result of high sustained levels of reactive species in ALD patients. The increase in catalase and superoxide dismutase activity suggested the formation of hydrogen peroxide and superoxide anion, respectively. Peroxisomes contain many of the cellular enzymes that generate hydrogen peroxide such as glycolate oxidase, urate oxidase and flavoprotein dehydrogenases involved in $-oxidation of fatty acids[16]. Recently, Biase et al. have used plasma low-density lipoprotein (LDL) as an indicator for oxidative stress in both ALD and AMN patients[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 substantial nigra[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[36]. Dopamine generates free radicals and hydrogen peroxide by auto-oxidation or through normal enzymatic processing by MAO[37]. Consequently, high levels of hydrogen peroxide are present in the substantial nigra.

It has also been suggested that the neuropathology of Huntington's chorea involves oxidative stress, although most of the evidence is indirect[36]. Post-mortem 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[28].

Evidence for the role of oxidative stress in Alzheimer's disease aetiology is accumulating.[38,40]. Various products of oxidation reactions, e.g. oxidised glutathione molecules, and mediators of oxidative stress, e.g. accumulation of free fatty acids, are found in brain of patients with Alzheimer's disease[39]. Basically, most of cellular macromolecules (DNA, protein, and lipids) can be found in an oxidised form in Alzheimer's disease brain tissue[39-40]. Other studies indicate that superoxide dismutase activity is decreased in Alzheimer's disease brain although these results are not substantiated in other studies.[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[42,43]. ALD may have more than one pathomechanism which explain the presence of different form 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[44]. Consequently, one of the useful neuronal rescue strategies could be the treatment with antioxidant agents[36,41,45].

Conclusion: 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.

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