Potential future therapies for X-ALD

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A few potential future therapies have been investigated in the following document. These include:

- Pharmacological intervention by Fibrates (Page 1)
- Modified Gene Therapy (Pages 2 - 3)
- Enzymatic Controls (Page 4)
- Antioxidant intervention (Pages 5 - 6)

Fibrate Drugs:


[Freely available online at: http://www.springerlink.com/content/p0819uu81w81518j/ ]

Within this paper, the researchers begin by describing that VLCFAs are primarily derived from endogenous synthesis via elongation of very long fatty acid (ELOVL) enzymes. Seven of these ‘elongases’ have been denoted in mammals (ELOVL1 - ELOL7). Pharmacological inhibition by Fibrate drugs (including Benzafibrate; BF), a class of amphipathic carboxylic acids which are used for a range of metabolic diseases, have been described to have potential to reduce VLCFAs in individuals with malfunctioning ABC proteins e.g. X-ALD sufferers.

A daily 400μM (micromolar) amount of the BF displayed reductions of up to 75% in C26:0 levels within human fibroblasts (a type of specialised skin cell) isolated from X-ALD patients.

The key researchers of this paper have suggested two possible means of how pharmacological intervention could have facilitated these results:

1. Indirect modification of other cellular processes, such as gene expression (how much of a particular gene is read and used to produce proteins, such as the elongase enzymes) via epigenetics for example. Epigenetics is a way the body can control what genes are expressed simply by attaching methane groups to particular areas of the DNA, a bit like putting a set of bollards in a road – it stops cars traveling along the road; methane groups block transcribing enzymes from traveling along that particular part of DNA.

2. Direct inhibition of the Elongase enzymes (ELOVL1 - ELOL7) themselves. This would mean that BF, or its metabolites (broken down BF products), fit into the active site of the enzymes blocking them, a bit like a key in a lock, and reducing synthesis of C26:0 Fatty acids.

OVERALL COMMENTS: The work described in the paper suggests that inhibition of VLCFA synthesis by pharmacological means could be a feasible treatment option for X-ALD. BF is a good candidate for this approach, with a proven safety profile for (long-term) use in humans. With a maximum daily dose of BF therapeutic levels might be reached in plasma. A small-scale proof of principle clinical trial is currently ongoing in the US to evaluate the effect in X-ALD patients, if this trial proves successful it may not be long before we see clinical trials involving BF in the United Kingdom.
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Modified Gene Therapy:


Allogeneic hematopoietic cell gene therapy has been shown to result in long-term benefits by arresting the progression of cerebral demyelination in boys with X-ALD. When performed at an early stage HCT is thought to be one of the only therapeutic approaches that can stabilize cerebral demyelination. However, it is not known as of yet if HCT can prevent, or rescue, adrenomyeloneuropathy in affected individuals.

This study described the use of a gene delivery vehicle - Lentiviral vectors. They have recently been adapted thanks to their ability to integrate into the genome (DNA) of non-dividing cells. (See image below)

![Lentiviral vector](http://en.wikipedia.org/wiki/File:Lentiviral_vector.png)

The above image displays how the defective gene, ABCD1 in CD34+ cells (a type of cell involved in the immune system) from patients with X-ALD, can be replaced by the functioning gene from healthy donor cells. CD34+ cells were taken from the X-ALD patients' blood, and essentially mixed with the Lentiviral vectors containing the un-mutated DNA within a laboratory setting (in vitro). The vector, being a particular type of virus, is able to transfer the healthy DNA section into the target cells genome via 'POL' enzymes (a type of polymerase enzyme).

The two patients in this study were aged 7 and 7.5, and both had older siblings who died of ALD. The cells that were denoted as having incorporated the new (healthy) DNA into their genomes
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were reintroduced into the patients. These altered cells would then be left to multiply and reintroduce some functional ABC proteins.

Follow-up tests were completed with both patients. Interestingly enough, their MRIs indicated that progression of cerebral myelinising lesions was arrested at 12-14 months after HCT. Furthermore, up to 36 months no additional lesions or changes in the MRI had been noted. These findings suggest that the production of functional ABC proteins may have reduced the build up of harmful VLCFAs, which may have reduced the neurological effects of these FAs.

OVERALL COMMENTS: This process, although tricky and with potential dangers, has been shown to be a potentially influential therapy for boys with X-ALD. However, this was a small-scale study, with only two participants (n=2). This means that what may have worked for the two patients tested, may not work for others. Additionally, other unknown factors may have contributed to the alterations in MRIs – the patient’s genomes may have contributed in some way to their altered cerebral demyelination? Furthermore, it would be sensible to suggest that longer post-HCT tests be performed to check if the changes in cerebral lesions were indeed a concrete change, not a ‘blip’ that may alter at a later date.

*Additional information on HCT can be found at the following link: http://www.x-ald.nl/treatment-options/hsct/*
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**Enzymatic controls:**


This study underlines the importance of a potent histone deacetylase (HDAC) inhibitor - Suberoylanilide hydroxamic acid (SAHA) in inducing the expression of various ABCD genes - producing ALD proteins, and normalizing peroxisomal beta-oxidation (the breakdown, or catabolism, of VLCFAs) in cultured human cells from X-ALD patients.

The cells used in this study (Fibroblasts and Astrocytes) were obtained from secure storage (Coriell Cell Repositories: www.ccr.coriell.org), after being donated by various X-ALD patients.

The researchers report that at the highest dose of SAHA (5μM) significantly increased ABCD2 gene expression by up to 11.8-fold in three days of drug administration in cultured fibroblasts (See page 1 for information on these cells). Additionally, a 10-fold increase in the expression of ABCD3 genes was also seen within fibroblasts.

Suberoylanilide hydroxamic acid was also denoted to significantly increase peroxisomal beta-oxidation in fibroblast cells, meaning there was an increase in VLCFA breakdown. Therefore, the levels of VLCFAs decreased, which would conceivably lead to a reduction or at least a slow-down of demyelination in the cerebrum.

Furthermore, SAHA reduced the production of Reactive Oxygen Species (ROS) in Astrocyte cells (a type of cell found only in the brain), as well as reducing the production of Tumour Necrosis Factor - alpha (TNF-α), a pro-inflammatory cytokine thought to influence cerebral demyelination.

**OVERALL COMMENTS:** SAHA has been shown to provide a favorable outcome in a variety of animal models of inflammatory disease conditions including LPS-induced endotoxemia, lupus, graft-versus-host disease, ischemia, TLR activation, septic shock and inflammatory hyperalgesia. Moreover, SAHA is presently in numerous clinical trials; more than 100 clinical trials with SAHA are in progress at the NIH (http://www.clinicaltrials.gov). This indicates that SAHA is a potential candidate drug for correction of the metabolic defect as well as the secondary neuroinflammatory disease in X-ALD.
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Antioxidant intervention:

As discussed in the previous section, oxidative damage by Reactive Oxygen Species (ROS) are thought to contribute towards the cerebral demyelination in X-ALD patients, due to the build up of VLCFAs in cells including Astrocytes.

A variety of antioxidant related therapies are out there, but these are two of which I think collectively display a positive take home message about the potential of antioxidant intervention as a therapy.


The study used mouse models, with the ABCD1 gene knocked out (removed) (ABCD1 models). The mice models displayed a variety of late onset neurological phenotypes with locomotor disability and axonal degeneration in the brain and spinal cord.

The researchers focused upon three antioxidants; N-acetyl-cysteine (NAC), alpha-lipoic acid (LA), and alpha-tocopherol. These three antioxidants were described as scavengers of VLCFA-dependant ROS, generated out of the body in laboratory conditions (in vitro).

The three antioxidants were administered (LA, and alpha-tocopherol in food – chow, and NAC in water) in a pre-clinical setting. This cocktail of antioxidants displayed three remarkable reversals in the mice models:

1) Oxidative stress and lesions to proteins reduced.
2) Signs of axonal degeneration reversed.
3) Locomotor impairment in a variety of physical/behavioural tests performed with the mice reduced also.

Therapeutic implications derived from this work could also be extrapolated to other diseases that share both axonal degeneration and oxidative stress as a main or early contributing pathogenic factor, e.g. motor neurone disease.

OVERAL COMMENTS: These three factors displayed that the impact of oxidative damage to the brain and spinal cord resulting from the build up of VLCFAs is a particularly major one. This also suggests that antioxidant intervention could be yet another potential therapy for X-ALD. However, this treatment would only appear to be a possible therapy in combination with other drugs that would reduce proinflammatory cytokines in patients with severe neuroinflammatory demyelination present.
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This was a six-month pilot study, whereby Valproic acid (VPA), a drug already used as a therapeutic agent for epilepsy, was administered both in body (in vivo) and out of body in a laboratory setting (in vitro). The drug has previously been shown to be able to freely cross the blood-brain barrier, i.e. diffuse from the blood circulatory system into the brain, a process that is very difficult to obtain. This gave the drug a big head start as a possible therapeutic agent involving X-ALD as a neurological disorder.

As described in the previous study on antioxidants, the researchers of this paper investigated X-ALD fibroblasts, which were cultured in the presence of VPA (2mM) for 2 and 12 days. A 2.8-fold increase in ABCD2 gene expression was seen in X-ALD fibroblasts after 2 days treatment. Also a 1.4-fold increase in ABCD3 expression was also denoted after treatment with VPA. In contrast, ABCD4 expression was not affected by VPA administration.

It was denoted that VPA did not alter C26:0 levels, nor C24:0. However, in the presence of VPA the production of C26:1w9, a monounsaturated VLCFA fell by over 40% - a big drop. This raises the possibility that the antioxidant Valproic Acid (VPA) might, at least partially, mediate the decrease in C26:1w9.

As the half-life (the time of the drug circulating in the body prior to excretion of breakdown) is very short, only 2.5 hours in rodents compared to 8-10 hours in humans an ex-vivo system rather than in-vivo was used. Whereby rodents were sacrificed and their hippocampus (part of the brain) finely sliced at 200 uM thickness and exposed to the VPA. A small number of human hippocampal tissue samples, acquired from epilepsy patients who had brain surgery, were also treated the same and exposed to VPA to see if ABCD2 expression increased.

ABCD2 expression increased in both rodent and human samples, but was higher in humans compared to the rodents, but this may have been due to interspecies differences.

OVERALL COMMENTS: VPA, although not effective upon C26:0 or C24:0, was seen to reduce C26:1w9 levels by an increased expression of ABCD2 and ABCD3. This suggests that the drug could be combined with other antioxidants in a cocktail method to not only increase the amount of functional ABC proteins, but also reduce the levels of harmful VLCFAs in the brain and spinal axons.

* Please note that all information documented has come from above referenced sources, none of the study outcomes nor the information they provide is owned by me, G. Metcalf, or ALD Life *