Dietary treatment as a basic concept in X-linked ALD/AMN

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**X-linked Adrenoleukodystrophy (X-ALD)**

- Most common peroxisomal disease and leukodystrophy (~1:20,000 newborns, combined incidence of male and female phenotypes)
- Inherited neurometabolic disorder caused by ABCD1 gene mutations (Xq28)
- Loss of function of peroxisomal ABCD1 transporter protein
- Diagnostic accumulation of saturated VLCFA's (> C22:0) in plasma and target organs (adrenals, CNS)

X-ALD basically is an inborn error of metabolism!
Human FA elongation pathways

- SFAs - MUFAs - PUFAs (n-3, n-6)
- FA partly essential, e.g. C18:2 (linoleic acid) or C18:3 (linolenic acid)
- FA elongation catalyzed by elongases ELOVL1-7, e.g. ELOVL1 elongates saturated and unsaturated C20-C26 acyl-CoAs
Where do VLCFA come from?

1. Adsorbed from food

2. Elongation from shorter fatty acids in endoplasmatic reticulum (ER)
VLCFA have essential physiological functions, e.g. myelin maintenance
Very long chain fatty acids incorporation in sphingolipids

Unique biological properties of C24-Sphingolipids in myelin membranes
- Effects on membrane fluidity
- Lipid microdomain formation
- Signaling across the membrane
- Cell death

2-HYDROXYLATED (2-OH) VLCFA IN THE MYELIN
catalyzed by the fatty acid 2-hydroxylase (FA2H)
- Mutations in the FA2H gene on chromosome 16 are associated with leukodystrophy with spastic paraparesis and dystonia (Edvardson et al., 2008)

What is the role of VLCFA incorporation in X-ALD?
Biochemical aspects

Main biochemical hallmark in ALD: Enhanced fatty acid elongation and insufficiently FA degradation

Accumulation of VLCFA

- Tissue specific incorporation of VLCFA, e.g. 39fold excess of C26:0 in wm phosphatidylcholine fraction
- Selectively enriched VLCFA in wm gangliosides
- Active demyelinating lesions: VLCFA containing cholesterol esters accumulating in invading monocytes/macrophages and activated microglia
  - Crystalline needles
  - Cellular stress
- High concentration of lyso-phosphatidylcholins
  - Induction of microglia apoptosis
  - Macrophage recruitment from periphery
  - Diagnostic marker for X-ALD (newborn screening)
VLCFA toxicity, mitochondrial dysfunction and dying back axonopathy in X-ALD
VLCFA toxicity and mitochondrial dysfunction

Working hypothesis

López-Erauskin et al, HMG 2013
Focal axonal degeneration in inflammatory diseases of the CNS: the role of mitochondria

Craner MJ, Nat Med 2011
Neuropathology in X-ALD mice

**ALD**
- Sciatic nerve
- 16 months
- Hypermyelination

**ALD**
- Spinal cord
- 21 months
- Focal axonal degeneration

Pujol et al, HMG 2002
Oxidative stress, axonal pathologies and mitochondria depletion are halted by pioglitazone in 18-month-old X-ALD mice

Pioglitazone halts locomotor disability in X-ALD mice: bar-cross test

Pathogenetic concept in X-linked Adrenoleukodystrophy

- ABCD1 gene defect
- VCLFA $\uparrow$
- Hormonal deficits
- Neurodegeneration
- Oxydative stress
- Energy failure
- Environment Modifier genes Epigenetic factors (ABCD2, SNP [CD1, Methionine...])

Phenotype expression
Axonal degeneration - Inflammatory demyelination
Abnormal accumulation of saturated very long chain fatty acids (VLCFA) is the principal biochemical abnormality in X-ALD [Igarashi et al., J Neurochem 1976].

Very long chain fatty acids (VLCFA)

Neurotoxic
Pro-inflammatory
Initiating oxidative stress
Inducing neuronal apoptosis
Background

- The VLCFA excess is greatest in those tissues that show the greatest pathological involvement (brain white matter, peripheral nerve, adrenal cortex, and testis) and VLCFA excess in brain co-localizes with the site of inflammatory lesions [Paintlia et al., Neurobiol Dis 2003]

- The increase of brain VLCFA is greatest in patients with the most severe phenotype [Asheuer et al., Hum Mol Genet 2005]

- VLCFA exposure in brain tissues is directly followed by an inflammatory reaction [Eichler et al., Ann Neurol 2008]

- VLCFA exposure to oligodendrocytes and astrocyte cultures is leading to mitochondrial dysfunction and cell death within 24 hours [Hein et al., Hum Mol Genet 2008]
Rationale for the normalization of VLCFA
(experimental animal experiences)

- Elevated VLCFA brain tissue levels from ABCD 1/2 knock-out mice in vitro and in vivo is followed by liberation of inflammatory mediators leading to inflammatory demyelination [Khan et al., 2010]

- VLCFA accumulation in fruit flyes (Bubblegum-Mutants) is leading to severe brain pathology which is reversed by LO treatment [Min et al, 1999]

- Over expression of ALDR followed by normalization of VLCFA corrects axonal pathology and clinical symptoms in ALD mice [Pujol et al.]

- Stimulation of alternative VLCFA degradation pathways is preventing neurodegeneration in ALD mice [Fourcade et al., 2009]
GTO/GTE Therapy


- Proposed pathogenetic effects of LO [Sassa et al., 2014]
  - Direct ELOVL1 inhibition by oleic and erucic acids (mixed inhibition)
  - Indirect ELOVL1 inhibition by C22:1-CoA (competitive inhibition)
  - Reduced Sphingomyelin levels with saturated C22 or C24 VLCFA

A mixture of oleic and erucic acids inhibits ELOVL1 activity.
Hypothesis

- VLCFA are toxic

- Toxicity of increased levels of VLCFA is prooven in vitro and various animal models (Brites et al., 2009)

- Normalization of the toxic VLCFA in X-ALD may prevent neurologic damage, even if does not reverse abnormalities in symptomatic patients.
GTO/GTE Therapy

ADRENOLEUKODYSTROPHY: COMPARISON OF GTE-GTO AND GTO OIL ON PLASMA C26:0 LEVELS

- GTE-GTO TREATED n=39
- GTO CONTROL n=15
- GTO TREATED n=16

C26:0 µg/ml (MEAN)

Early studies


- Critical points: inadequate measures, small numbers with heterogeneous phenotypes, short and inadequate controlled treatment periods, NON CONTROLLED
Evaluation of the preventive effect of glyceryl trioleate-trierucate ("Lorenzo’s Oil") therapy in X-linked adrenoleukodystrophy: Results of two concurrent trials

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Evaluation of the preventive effect of glyceryl trioleate-trierucate ("Lorenzo’s Oil") therapy in X-linked adrenoleukodystrophy: Results of two concurrent trials

- 105 presymptomatic patients, treated before age 6
- Mean duration of therapy
  - USA group: 2.6 years (range 0.3 to 10.7)
  - European group: 4.3 years (range 1-10.4 years)
- Method 1: Onset of symptoms in treated patients compared to a natural history cohort
- Method 2: Correlation between lowering of plasma 26:0 levels, neurologic outcome and MRI progression
  - Group 1: Mean annual C26:0 level within 2 SD of normal mean for two or more years
  - Group 2: failed to lower C26:0 to this extend or duration

Evaluation of the preventive effect of glyceryl trioleate-trierucate ("Lorenzo’s Oil") therapy in X-linked adrenoleukodystrophy: Results of two concurrent trials

- Later onset of disease in LO treated groups (ITT)
- LO treatment clearly not fully preventive
Evaluation of the preventive effect of glyceryl trioleate-trierucate ("Lorenzo’s Oil") therapy in X-linked adrenoleukodystrophy: Results of two concurrent trials

Age adjusted hazard for development of neurological abnormality

- USA: group 1 risk 23% of group 2 (p=0.013)
- Europe: group 1 risk 13% of group 2 (p=0.006)

Hazard of developing neurologic abnormality increased with age at which therapy was started

Onset of symptoms

Onset of MRI changes
Conclusion

Lorenzo Oil therapy in neurologically uninvolved boys with ALD who are less than 6 years old delays the onset of neurologic and MRI abnormality provided that it is accompanied by substantial and prolonged lowering of plasma C26:0 levels
GTO/GTE Treatment:
Long-term Follow-up in AMN

Patients and Methods

- „pure“ AMN (no lobar brain involvement in MRI, brain stem tract involvement not excluded, Loes < 2)
- Calculation of an annual clinical progression rate before treatment using a disease specific scoring system (AACS)
- Comparison of the pre-treatment progression rate with changes in AACS under treatment
- Minimum individual treatment period of 2 years, minimum mean treatment period of 5 years
GTO/GTE Treatment: Long-term Follow-up in AMN

Treatment, study protocol

- C26:0 low diet (<3g C26:0/day)
- GTO/GTE 1ml/kg body weight (initial dosage)
- Titration according to plasma VLCFA levels (goal: normal range of C26:0)
- Monthly VLCFA measures (first year), 3-monthly from year 2
- Yearly follow-up investigations (clinical, MRI, neurophysiology, neuropsychology)
GTO/GTE Treatment: Long-term Follow-up in AMN

Clinical progression of untreated patients with AMN

\[ y = 0.4103x \]

- \(-1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10\)
- \(-1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14\)

- e.g. wheelchair bound, loss of bladder and sensory functions
- e.g. walking with assistance, moderate sensory and bladder dysfunction
- e.g. walking independently with slight difficulties, no sensory, no bladder dysfunction
GTO/GTE Treatment: Long-term Follow-up in AMN

AMN Compliant - Group (VLCFA normalized, n=44)
AACS - Changes between Annual Follow-up and Baseline

\[ y = 0.0875x + 4.2582 \]
Dietary Treatment (general principles)

- Balanced, fat-modified dietary treatment to lower VLCFA
- Eventually combined with GTO/GTE treatment
- Adequate energy supply
- Avoid rapid changes of body weight
- Relation of nutrients:
  - 40% carbohydrates
  - 40% fat
  - 20% protein
- Monthly plasma VLCFA evaluation
- Supporting tools: continuous support by dietitians, list of recommended foods, AMN cook-book, eating protocols calculated by dietitian, feedback to pts.
Dietary Treatment

- **Fat** (mainly animal fat (pork, goose, eggs), olive oil, rapeseed oil, mustard oil)
  - 60-63% mono-unsaturated fatty acids
  - 15% poly-unsaturated fatty acids
  - 20% saturated fatty acids
  - 2-5% medium chain triglycerides

- **Protein**
  - 50% from animals (pork, poultry, certain fish)
  - 50% from plants (grain [no wholemeal], potato, vegetables)

- **Carbohydrates**
  - Mostly vegetables
  - Grain
  - Limited amounts of fruit (max. 300g/day, low fructose)
Dietary Treatment  (current status)

- 27 patients included
  - 17 males, 10 females
  - Most patients with good compliance
  - Up to 36 month of treatment
  - Most patients (males) need additional GTO/GTE treatment to normalize VLCFA
C26:0 (mean)
LO Dosage

![Bar chart showing LO dosage with Summe LO and LO Dosis/Pat categories.]
patient-example 1

LO Dosage
C26:0

0,10
1,00
10,00
100,00

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

patient-example 2

LO Dosage
C26:0

0,10
1,00
10,00
100,00

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
"Take home messages"

- Normalization of VLCFA appears to be a basic therapeutic principle in X-ALD to prevent neurodegeneration
- Diet and Lorenzo’s oil treatment are normalizing VLCFA within 4-6 weeks,
- Pre-clinical and clinical data are highly suggestive in favor of dietary treatment; however RCT’s are urgently missing
- Dietary treatment is NOT fully preventive, but it is something that YOU can do to influence YOUR own personal situation.
Thank you for your attention!