The Role of Organic Acids in the Diagnosis of Peroxisomal Biogenesis Disorders

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Peroxisomes

- Small sub-cellular organelles
- Present in all eukaryotic cells
- Abundant in tissues actively involved in lipid metabolism
  - Liver
  - Kidney
  - Nervous tissue
Functions of Peroxisomes

- Fatty acid β-oxidation
- Fatty acid α-oxidation
- Ether-phospholipid biosynthesis
- H₂O₂ metabolism
- L-pipecolate degradation
- Glutaryl-CoA metabolism
- Glyoxylate detoxification
- Isoprenoid biosynthesis
Peroxisomal Biogenesis

• Three steps:
  – Formation of lipid bilayer
  – Incorporation of membrane-bound peroxisomal proteins
  – Import of matrix proteins into the peroxisome

• \textit{PEX} genes encode proteins required for assembly of the peroxisomal membrane and support the import of matrix proteins

• Protein products known as peroxins
Peroxisomal Disorders

- Single peroxisomal protein deficiencies
- Peroxisomal biogenesis disorders:
  - Rhizomelic Chondrodysplasia Punctata (RCDP) phenotype
  - Zellweger Spectrum:
    - Zellweger syndrome (ZS)
    - Neonatal adrenoleukodystrophy (NALD)
    - Infantile Refsum disease (IRD)

Zellweger syndrome

- Presents at birth
- Reduction or absence of peroxisomes
- Clinical phenotype:
  - Craniofacial dysmorphism
  - Hypotonia
  - Impaired hearing/eye abnormalities
  - Psychomotor retardation, neonatal seizures
  - Liver disease
  - Calcific stippling of epiphyses
  - Renal cysts
- Death usually occurs within 6 months
Zellweger syndrome (2)

• Biochemical phenotype:
  – Plasma: increased very long chain fatty acids (VLCFA), phytanic acid, pipecolic acid, and bile acid intermediates DHCA and THCA
  – Erythrocytes: reduced plasmalogen synthesis
  – Fibroblast cultures: reduced dihydroxyacetone phosphate acyltransferase (DHAPAT) activity
  – Urine: increased pipecolic acid and bile acid intermediates

• Diagnosis: abnormal plasma VLCFA levels, confirmed by DHAPAT activity
Problems with Diagnosis of PBD

- Plasma VLCFA are not part of routine ‘metabolic screen’ in most metabolic laboratories
- Clinicians unfamiliar with rare disorders may not request VLCFA examination
- Urine most commonly submitted specimen type for metabolic screening

Therefore patients with an undiagnosed PBD may be missed when being screened for a metabolic disease
Role of Organic Acids

- GC-MS analysis of urinary organic acids commonly included in the routine ‘metabolic screen’
- Characteristic organic aciduria of PBDs has been reported, showing increased excretion of:
  - 3,6-epoxydicarboxylic acids (C10, C12, C13, C14)
  - Odd-chain C7 – C15 dicarboxylic acids
  - 2-hydroxydecanedioate
  - Saturated and unsaturated C6 - C10 dicarboxylic acids
  - C10:C6 and C8:C6 dicarboxylic acid ratios >1
  - 4-hydroxyphenyllactic and 4-hydroxyphenylacetic acid

Aim of Study

• To look for the presence of characteristic metabolites and other features of an organic acid profile in patients with a PBD as previously reported

• To identify the mass spectra of relevant metabolites for addition to the GC-MS searchable library, to allow routine identification of these metabolites in patient samples.
Methods

• Urine from 14 patients with various peroxisomal disorders was examined:
  – 8 Zellweger Syndrome
  – 2 Infantile Refsum Disease
  – 2 X-linked Adrenoleukodystrophy
  – 1 Pseudo-Zellwegers
  – 1 Refsum’s Disease

• Urine from 20 patients with no specific abnormality on urinary organic acids analysis was also examined

• GC-MS analysis of urine organic acids was carried out on all samples
Results:
Organic Aciduria of PBD
Odd-chain dicarboxylic acid: 3,6-epoxytetradecanedioate

Saturated & unsaturated even-chain dicarboxylic acids:
- C6
- C8:1
- C8
- C10:1
- C10

Even-chain dicarboxylic acids:
- 2-OH decanedioate
- 3-OH decanedioate

4-OH phenylacetic acid, 4-OH phenyllactic acid

Normal
Mass Spectrum of 3,6-epoxytetradecanedioate

Scan 3531 (24.268 min): 850.0

Scan 3531 (24.268 min): 850.0

TMS COO TMS

m/z: 416
Results

• 8/10 patients with PBDs showed increased excretion of:
  – 3,6-epoxydicarboxylic acids (mostly C14)
  – 2-hydroxydecanedioate

• 3,6-epoxytetradecanedioate:
  – Specificity: 100%
  – Sensitivity: 80%

• All types of peroxisomal disorders showed elevated levels of odd-chain dicarboxylic acids (mostly C7 and C9)
Results

• 7/10 patients with PBDs showed C10:C6 and C8:C6 dicarboxylic acid ratios of >1
  – Specificity: 100%
  – Sensitivity: 70%

• Increased levels of 4-hydroxyphenyllactic acid and 4-hydroxyphenylacetic acid were present in patients with a PBD or pseudo-Zellwegers
A few weeks later…

• Urine from a 1 month old baby analysed
• Clinical details “failure to thrive”
• Urine organic acids:
  – Increased 2-hydroxydecanedioate
  – Increased 3,6-E14DA
• Peroxisomal disorder suspected
• Plasma for VLCFA already received, which confirmed diagnosis of a PBD
Conclusions

• Urinary organic acids can be a useful indicator to the diagnosis of a PBD

• This particular organic acid profile should alert the laboratory to the possibility of a PBD and prompt appropriate investigations, including VLCFAs.

• Awareness of the characteristic organic aciduria of a PBD may improve detection of these conditions in an initial metabolic screen
References


www.humpath.com/IMG/jpg/mitochondria_peroxisome_hepatocyte_04-2.jpg

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