Organic Acids

The basics

Donna Fullerton, Nottingham City Hospital
What are organic acids?

- Organic compounds with acidic properties

- Most common organic acids are carboxylic acids whose acidity is attributable to the carboxyl group (COOH)

Carboxylic organic acids:

- Low molecular weight water soluble acids
- Ninhydrin negative
- Compounds detectable by urinary organic acid analysis
Where do they come from?

Organic acids are the end products and intermediates of a wide range of metabolic pathways.
How do we measure organic acids?

Gas-Chromatography Mass Spectrometry (GC-MS)
Organic Acid Analysis (1)

- **Extraction**

  - A volume of urine (adjusted for creatinine concentration) is acidified and then saturated with sodium chloride. Acidification converts polar organic acids to non-polar organic acids which have greater affinity for the ethyl acetate phase, hence maximising organic extraction. The sodium chloride reduces the solubility of the organic acid aqueous phase (salting out), further aiding extraction.

  - The organic acids are solvent extracted using ethyl acetate and diethyl ether.

  - After evaporation of the ethyl extracts the organic acids are derivatised.

  - The organic acids are derivatised to form trimethylsilyl (TMS) esters using BSTFA (N,O-Bis(trimethylsilyl)trifluoroacetamide) and pyridine. Derivatisation converts the unstable, low volatile organic acids into stable, volatile TMS derivatives which are suitable for separation by gas chromatography.
Organic Acid Analysis (2)

- **Gas Chromatography-Mass Spectrometry (GC-MS)**

  - The sample is pumped through a capillary across a high voltage and at a high temperature. This causes the liquid to evaporate into smaller droplets, until the charge becomes so great the electrons repel one another and undergo a cumbic explosion forming gaseous ions.

  - The gaseous ions interact with a column and are separated according to size. The smaller, volatile ions interact less with the column and are eluted first.

  - The gaseous ions enter the quadrupole mass filter of the mass spectrometer. The quadrupole is formed of 4 parallel rods in a strong vacuum to which fixed DC and alternating RF voltages are applied.

  - Under specific conditions, ions of a specific mass-to-charge (m/z) ratio will oscillate through the quadrupole to the detector. The resultant chromatogram is compared to a library of known compounds.
GC-MS

Organic acid extracts

Quadrupole of a Mass-Spectrometer
Organic Acid Disorders

- A block in the breakdown of an organic acid can lead to its accumulation in the cell and its elevation in plasma and urine. The first transamination step and second dehydrogenation step of amino acid catabolism generate organic acids.

- Often this leads to a metabolic acidosis but this is not always the case. It may also be associated with and combination of hypoglycaemia, hypocalcaemia, lactic acidaemia, hyperglycinaemia and hyperammonaemia. In some disorders one or more amino acids may also be elevated (eg Maple Syrup Urine Disease).

- Severe forms will present acutely in the neonatal period whereas other forms may present chronically over a long period of time sometimes with acute episodes.

- There may be multisystem involvement or the brain may be the only organ affected.

- Some of the more common organic acid disorders are discussed in the subsequent slides.
Propionic and Methylmalonic Aciduria

Propionic and Methylmalonic acids are formed from the catabolism of branched chain amino acids, odd chain fatty acids and cholesterol. They are ultimately converted to succinyl CoA which feeds into the Kreb’s Cycle. Defects in any of the steps below result in their accumulation.

1. **Propionyl CoA**
   - **Propionyl CoA Carboxylase**

2. **D-Methylmalonyl CoA**
   - **Methylmalonyl CoA Racemase**

3. **L-Methylmalonyl CoA**
   - **Methylmalonyl CoA Mutase**

4. **Succinyl CoA**
Clinical Presentation

• The more severe acute forms of Propionic aciduria (PA) and Methylmalonic aciduria (MMA) have similar clinical presentations:

• Neonatal presentation
  - Ketoacidosis
  - Vomiting
  - Hypotonia
  - Hypoglycaemia
  - Lethargy

• Infancy
  - Failure to thrive
  - Episodic illness associated with illness
  - Reyes Syndrome like episode
  - Convulsions associated with hypoglycaemia

• Other presentations
  - Chronic progressive neurological disease
  - Ataxia, metabolic stroke, extrapyramidal signs (abnormal movements)
Common Laboratory Findings in PA/MMA

- **Hypoglycaemia**
  - caused by inhibition of pyruvate carboxylase and the transmitchondrial malate shuffle by methylmalonyl CoA

- **Hyperammonaemia**
  - caused by inhibition of carbamoyl phosphate synthase by Propionyl CoA

- **Hypocalcaemia**

- **Elevated plasma lactate (and secondarily plasma alanine)**
  - with a high anion gap and a metabolic acidosis

- **Neutropaenia**

- **Increased plasma/urine glycine**
  - caused by inhibition of the glycine cleavage enzyme.
Laboratory Tests for the Diagnosis of Propionic Aciduria

Propionic aciduria is caused by a defect in propionyl CoA carboxylase (PCC). Propionate metabolism is blocked in the absence of PCC activity and intermediary products accumulate. These are detected by:

Urine Organic Acid Analysis

- $\text{3OH-propionate}$
- Methylcitrate
- Propionylglycine
- Tiglyglycine

Blood Acylcarnitine Analysis

- Increased Propionyl-carnitine (C3)
- Low free carnitine levels secondary to carnitine sequestration.

Confirmatory Tests

Assay of $14C$-propionate incorporation into cultured fibroblasts or of propionyl CoA carboxylase in cultured fibroblasts or leucocytes.
Organic Acid Trace of Propionic Acidaemia

Increased excretion of 3OH propionate, Tiglyglycine and methylcitrate
Treatment of Propionic Acidaemia

Treatment of acute attacks involves using sodium benzoate to remove ammonia, correction of the metabolic acidosis, preventing propionate production by stopping protein intake and giving glucose and insulin and removing propionate by giving carnitine.

Long Term treatment consists of a diet restricted in branched chain amino acids (the main source of propionate in the diet), preventing carnitine depletion by giving carnitine, giving metronidazole to stop gut bacteria producing propionic acid and sodium benzoate to prevent hyperammonaemia. In certain patients where management is difficult liver transplantation may be an option.

Despite treatment complications may occur such as mental retardation, movement disorders, osteoporosis, cardiomyopathy and pancreatitis.
Methylmalonic aciduria is caused by a defect in the activity of methylmalonyl CoA mutase. However in the majority of cases it is not the methylmalonyl CoA mutase enzyme itself that is deficient but its cofactor adenosylcobalamin synthesised from Vitamin B12. Propionyl CoA carboxylation is reversible and so propionate metabolites can also accumulate in methylmalonic aciduria. These are detected by:

- **Urine Organic Acid Analysis**
  - Methylmalonate
  - 3OH propionate
  - Methylcitrate

- **Blood Acylcarnitine Analysis**
  - Raised propionylcarnitine but low free carnitine levels
Increased excretion of MMA, 3OH-propionate and methylcitrate
Methylmalonic Aciduria and Cobalamin

Propionyl CoA

D-Methylmalonyl CoA
  - Propionyl CoA Carboxylase

L-Methylmalonyl CoA
  - Methylmalonyl CoA Racemase
  - Methylmalonyl CoA Mutase

Succinyl CoA
  - Adenosylcobalamin (AdoCbl)

Cobalamin
  - Methylcobalamin (MeCbl)

Homocysteine ➔ Methionine
The common causes of increased urine and plasma methylmalonic acid are as follows:

**Acquired**

**Vitamin B12 Deficiency**
- Secondary to dietary deficiency. Sometimes seen in breastfed children of Vegan parents. Mild deficiencies may not show anaemia or neurological symptoms.

**Inherited**

NB Serum vitamin B12 concentrations are normal.

**Methylmalonic Aciduria**
- **Methylmalonic Mutase Deficiency:**
  - Complete deficiency (mut⁺)/Partial deficiency(mut⁻)
- **Adenosylcobalamin Synthesis defects**
  - Mitochondrial Cobalamin reductase deficiency (cblA)
  - Mitochondrial Cob(I)alamin adenosyltransferase deficiency (cblB)

**Combined Methylmalonic Aciduria and Homocystinuria**
- Defects in Adenosyl and Methylcobalamin synthesis
  - CblC, CblD and CblF
Additional Laboratory Tests

- Confirmation of the diagnosis can be made by measuring the incorporation of radiolabelled propionate into fibroblast cell protein and Vitamin B12 responsiveness can be tested by adding this to the cells.

- Further delineation of the primary defect is more difficult. Methylmalonyl CoA mutase can be assayed directly although these account for only a small proportion of cases. Since direct enzyme assays for the enzymes of adenosylcobalamin and methylcobalamin biosynthesis are difficult, the usual approach is to determine the affected gene by fusing the fibroblasts with known patient cell lines and seeing if they normalise activity (complementation analysis). Once identified then mutation screening can be performed.
Treatment of Methylmalonic Aciduria

• Acute illness

The treatment is identical to that for propionic aciduria

• Long term treatment

In cases of isolated methylmalonyl malonic aciduria the patients are put on a diet restricted in branch chain amino acids. Patients with combined methylmalonic aciduria and homocystinuria also have to have a methionine and threonine restricted diet. As with propionic acidaemia carnitine and metronidazole are used. Betaine may also be given in the case of homocystinuria to help reduce the homocysteine concentration by the action of a liver specific enzyme that can convert homocysteine to methionine using betaine as its methyl donor.

Patients with partial defects in adenosylcobalamin synthesis sometimes respond to supraphysiological doses of Vitamin B12. Responding patients are continued on this often with additional folate supplements. As one would expect patients who respond have a better prognosis. However, despite this they can develop developmental delay, metabolic strokes and learning difficulties.

Non-responding patients, despite treatment, can develop renal problems secondary to the toxic effects of methylmalonic acid on the kidney.
Glutaric Acidaemia (Type I)

• Autosomal recessive condition caused by a deficiency in the enzyme glutaryl-CoA dehydrogenase which is involved in lysine/tryptophan metabolism.

```
Tryptophan
    ↓
    ↓
    ↓
2-Amino, 3-Carboxymuconic Semialdehyde (Picolinic acid)
    ↓
    ↓
Aminomuconic Acid  Quinolinic Acid
    ↓                    ↓
Glutaryl-CoA  Nicotinic Acid
    ↓                    ↓
Crotonyl-CoA  Nicotinamide
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Glutaryl-CoA Dehydrogenase
Clinical Presentation of Glutaric Aciduria Type 1

- Incidence 1:30,000 – 1:100,000
- Macrocephaly at birth
- Acute encephalopathic episodes - typically during period of illness
- Sudden onset of hypotonia and severe movement disorders following an acute episode

Neurological findings
- Often an initial period of normal development with “soft” neurological signs eg. Irritability/jitteriness
- Acute episodes associated with striatum destruction
- Frontal/temporal atrophy
- White matter changes may be observed
- Basal ganglia changes may be evident after an encephalopathic episode
Laboratory Findings in Glutaric Aciduria Type 1

NB Metabolic abnormalities may be fluctuating and inconsistent. In acute episodes there may be hypoglycaemia, metabolic acidosis and hyperammonaemia.

- **Urine Organic Acids**
  - Increased glutaric acid
  - Increased 3-hydroxyglutarate
  (NB. A small group of patients may not show increased levels of glutaric acid and only trace levels of 3OH glutarate. Glutarate and 3OH glutarate can be non-specifically elevated due to bacterial contamination)

- **Blood Acylcarnitines**
  - Increased Glutaryl carnitine
  - Free carnitine levels may be low

- **Enzyme studies (Definitive test)**
  - Glutaryl CoA dehydrogenase activity in cultured fibroblasts
  (Typically <5-10% normal activity level in affected individuals)

- **Mutation Analysis**
  - No common mutations except in certain ethnic isolates.
Organic Acid Trace of Glutaric Aciduria Type I

- Glutarate
- 3OH Glutarate
- Heptadecanoate IS
Treatment of GA1

Acute Episodes
The hypoglycaemia, metabolic acidosis and hyperammonaemia need to be managed and an emergency protocol is required to deal with these situations.

Long Term Management
The aim of dietary treatment is to reduce the accumulation of the presumed toxic agent glutaric acid by reducing its rate of formation. To this effect patients can be put on a low protein diet which specifically restricts lysine and tryptophan. Alternatively they can be put on a lysine free diet however they will need tryptophan supplements for protein biosynthesis.

Patients are also given carnitine to avoid carnitine depletion secondary to the excretion of glutaryl carnitine.
Questions

Which of these biochemical abnormalities occur with the following disorders:–

Glutaric Aciduria

Propionic Aciduria

Methylmalonic Aciduria

1. Elevated urine 3-hydroxypropionate
2. Elevated urine methylcitrate
3. Elevated urine 3-Methylmalonic acid
4. Elevated 3-hydroxyglutarate
5. Hyperammonaemia
6. Hyperlactic acidaemia
7. Hypoglycaemia
8. Hyperglycinaemia
9. Low plasma carnitine
Answers

- Glutaric Aciduria
  4, 5, 6, 7 & 9

- Propionic Acidaemia
  1, 2, 5, 6, 7, 8, & 9

- Methylmalonic Aciduria
  1, 2, 3, 5, 6, 7, 8 & 9