Metabolic studies on Hif-p4h-2 hypomorphomic mice

The three Rs in metabolism research

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Hypoxia response

Hypoxia inducible factor prolyl 4-hydroxylases (HIF-P4Hs) act as cellular oxygen sensors.
Hypomorph Hif-p4h-2\textsuperscript{gt/gt} mice express different Hif-p4h-2 levels in different tissues.
Statistics of *Hif-p4h-2*\(^{gt/gt}\) mice

453 born between June – December 2015

- 90 mice (20%) \(gt/gt\)
- 129 mice (28%) \(wt\)
- 234 mice (52%) \(gt/wt\)

All \(gt/gt\) and \(wt\) used for experiments

20 females, 15 males used for breeding
Experiments

High fat diet
• To study changes in glucose metabolism
• Mice are fed a standard rodent diet (18% kcal fat) or an high-fat diet (42% kcal fat) for 6 weeks

FG-4497 treatment
• Small molecule compounds that inhibit HIF-P4Hs are being developed for the treatment of e.g. anemias
• FG-4497, 60 mg/kg, is administered orally three times a week

Glucose tolerance test
• Mice that had fasted for 12 h are anesthetized with fentanyl/fluanisone and midazolam
• Fasting blood glucose measured
• The mice are then injected i.p. 1 mg/kg glucose and blood withdrawn at 15, 30, 60 and 120 min post injection for blood glucose
Alcoholic fatty liver disease - study

• Protection against steatosis and fatty liver disease with HIF-P4H-2 inhibition?

• Genetic model (\textit{Hif-p4h-2}^{gt/gt} mice and wt littermates)
  – three weeks on 5% ethanol diet

• Pharmacologic model (FG-4497) with wild-type mice
  – four weeks on 5% ethanol diet
  – Simultaneous treatment with FG-4497 or vehicle
Metabolic cage measurement

- Drinking and feeding behavior
- Metabolic performance (O$_2$ consumption/CO$_2$ production, respiratory exchange ratio and heat production)

For more information please visit our web page at:
http://cc.oulu.fi/~fysiowww/KHHmpr.html
Animal welfare

Analgesia
  – Depends on the experiment

Constant monitoring (daily, several times per day if needed)

Higher room temperature post-operation (24-25°C)

Housing in single cages/with other animals

Humane endpoints
  – Overall appearance (dehydration, abnormal posture, condition of fur),
    movement, behaviour, eating, weight loss
  – If problems arise, animal is treated (hydration, antibiotics)
  – If no improvement, animal is sacrificed
The 3Rs

Replacement
  – We always use cell culture experiments when feasible
  – E.g. silencing of HIF2α with siRNA to splenocytes in cell culture compared to AAV to mice

Reduction
  – Collection of all tissues instead of one when ever we sacrifice animals
  – Refinement of methodology – smaller variation, less animals needed to reach statistical significance in results
  – Starting with 4+4 or 6+6, adding more animals if needed

Refinement
  – Pain medication
  – Experienced personnel: not everybody does everything but the experienced one does
Importance of constant environment – an example when something went wrong
Protection against obesity and metabolic dysfunction

• Can HIF-P4H-2 inhibition be used to treat obesity and its consequences?
• *Hif-p4h-2*<sup>gt/gt</sup> mice were leaner and had less adipose tissue and smaller adipocytes
• They also had improved glucose tolerance
• Mice were protected against hepatic steatosis
• ⇒ HIF-P4H-2 inhibition may not only protect against the development of obesity and its consequences but also reverse these conditions

![Glucose tolerance of 1-year males](image)

Rahtu-Korpela et al. 2015 Diabetes