Metabolomics of hay fever and the pathophysiology of sneezing

Klaus M. Weinberger, Ph.D.

"Creating knowledge for health"

www.biocrates.at
Simple causal relationships...

Early spring in Austria

Disabled speaker

Early spring in Austria
Targeted metabolomics in pharmacodynamics and toxicology

Klaus M. Weinberger, Ph.D.
Metabolomics in systems biology

- Functional end-point of genetic information
- Mirroring external influences by:
  - (Mal-)nutrition
  - Physical activity
  - Medication

Enzymatic activity
Metabolites
Linking metabolites to enzymes and pathways

**Carb-P** → **Cit** → **ASS** → **Asp**

**OCT** → **NOS** → **NO** → **Argsucc** → **Fum**

**Orn** → **Arg** → **Urea**
Biomarker technologies

Discovery

Profiling Identification

Number of parameters

Validation

Identification

Targeted Quantitation

Number of samples

Application

NMR

GCxGC/MS

LC-MS (full ion scan)

LC-MS/MS

FIA-MS/MS

Precursor ion and neutral loss scans

LC-MS/MS

FIA-MS/MS

Multiple reaction monitoring

BIOCRATES life sciences
Integrated metabolomics platform

Sample preparation
- Automated separation and derivatization
- Solid phase extraction
- Alliance with Hamilton Robotics

Analytics
- Identification
- Quantitation
- QA/QC
- Alliance with Applied Biosystems / MDS Sciex

BioInformatics
- Technical validation
- Statistical analysis
- Data visualization
- Biochemical interpretation
- Partnership with GeneGo

LIMS/Database

BioBank
- Clinical & experimental samples
- Diagnoses & lab data
Analytical portfolio

- Primary and secondary amines
  - proteinogenic and non-proteinogenic amino acids
  - alkylated and nitrated amino acids
  - biogenic amines and polyamines
- Acylcarnitines and free carnitine
- Reducing mono- and oligosaccharides
- Phospho- and glycolipids
  - PC, PE, PS, PG, PA, PI(P), Cer, SM, ...
- Eicosanoids & other oxidized fatty acids (COX, LOX activity; non-enzymatic oxidation)
  - Prostaglandins, prostacyclins, thromboxanes
  - Prostaglandin D2, HETE, HODE, ...
- Bile acids & their conjugates
- Energy metabolism intermediates
  - Phosphorylated sugars
  - Nucleotides
  - Mono-, di-, trivalent organic acids

Routine quantitation of > 1000 annotated metabolites covering main areas of intermediary metabolism
Areas of application

- **Basic research**
  - Beyond genomics, transcriptomics and proteomics in cell biology, oncology, inflammation, etc.

- **Agricultural & nutrition industry**
  - Plant intermediary metabolism
  - Health effects of functional food
  - Nutrigenomics

- **Clinical diagnostics & theranostics**
  - Proof-of-concept in neonatal screening
  - Early diagnosis and accurate staging of complex diseases
  - Specific monitoring of therapeutic effects

- **Pharmaceutical R&D**
  - Drug metabolism / Pharmacokinetics
  - Safety / Tox
  - Pharmacodynamics / Efficacy
### Metabolites give insight at different levels

| I. Markers for activities of single enzymes | Carnitine palmitoyl transferase I  
Stearoyl-CoA desaturase  
NO synthase |
|-------------------------------------------|--------------------------------------------------|
| II. Direct multiparametric markers         | Lipid elevation/ lowering; subsets!  
Metabolic control / Insulin sensitivity  
Inflammation mediators  
Tubular dysfunction |
| III. Multiparametric surrogate markers     | Gluconeogenesis / Glycolysis  
Glycogen synthesis / Glycogenolysis  
Tissue damage and apoptosis  
Oxidative stress |
| IV. Mode of action markers                | Lipid signalling (PIs, PIPs, Ceramides, S-1-P etc.)  
Regulatory metabolites (ADMA/ SDMA, F-2,6-BP) |

**Additional mention:**
- **Lipid signaling** (PIs, PIPs, Ceramides, S-1-P etc.)
- **Regulatory metabolites** (ADMA/ SDMA, F-2,6-BP)**
Use case: puromycin-induced nephrotoxicity

Study design: 4 cohorts of 6 Sprague-Dawley rats

I. Controls only vehicle
II. 10 mg/kg no histopathology
III. 20 mg/kg moderate nephrosis
IV. 40 mg/kg ESRD after two weeks

Plasma and urine at 4 timepoints: Days 3, 7, 14, and 22

Correlated with histopathology, pathophysiology, expression profiling, and proteomics
General tissue damage

- Dose- and time-dependent increase in plasma acylcarnitine concentrations
- No preference for certain chain length, no depletion of free carnitine
- Established biomarker of tissue leakage and - particularly - mitochondrial damage
No overt hepatotoxicity
Oxidation of polyunsaturated fatty acids

12(S)-HETE
12-Lipoxygenase
15(S)-HETE
15-Lipoxygenase

Arachidonic acid
Dose-dependant cyclooxygenase activation

- Increased ratios of prostaglandins & thromboxanes to arachidonic acid
- PGE2 pro-diuretic
- PGE2 and TXB2 associated with proteinuria
**SDMA levels in puromycin-induced nephrosis**

- Plasma markers of kidney damage
  - Higher predictive value than ADMA
  - Reportedly lower SDMA clearance in rat remnant kidney model not observed here
  - Increased activity of protein arginine N-methyltransferase II

**Chart:**
- Plasma and Urine SDMA levels
- Comparison of ADMA and SDMA levels
- Structures of L-Arginine, N^\omega^\text{-Monomethyl-L-Arginine (L-NMMA)}, N^\omega^\text{-Dimethyl-L-Arginine (ADMA)}, and N^\omega^\text{-N^\omega^\text{-Dimethyl-L-Arginine (SDMA)}}
Time course in low-dose cohort
Time-dependent lysinuria

- No histopathological findings in 10 mg/kg group
- Separate resorption system for dibasic amino acids (Arg, Orn, Lys)
- Effects on Arg and Orn less pronounced (Cave: role in urea cycle)
Study summary

- Direct toxicity / tissue damage
  - Acylcarnitine leakage and release of membrane phospholipids
  - Activation of phospholipases
  - No overt hepatotoxicity

- Moderate polyuria and specific tubular dysfunction

- Inflammation / oxidative stress
  - Dose-dependant activation of COX, 12-LOX and 15-LOX
  - Increase of pro-inflammatory, pro-diuretic eicosanoids
  - No indication of systemic oxidative stress

- Other markers
  - Time-dependent moderate ketosis
  - De-repression of NO synthase
  - Elevated plasma SDMA, in this model also elevated urine levels
  - Depletion of plasma tryptophan, but mainly conversion to serotonin (instead of kynurenine); mediator for vasoconstriction in kidney
### Concerns for clinical studies

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Observed effects significantly greater than ‘normal’ biological variability?
Urea cycle in mild metabolic syndrome

- Urea cycle enzymes and intermediates are shown.
- Key components include Asp, ASS, Cit, Urea cycle enzymes, and Arg succ.
- The pathway shows how these components interact within the cycle.
Urea cycle alterations in metabolic syndrome

- Highly significant and specific differences even in small clinical study (n = 100)
- Ratios of metabolites and pathway mapping yield even clearer separation
Vision for drug development

Molecular pathophysiology

Evidence-based drug development

Molecular pharmacodynamics

Clinical chemistry

Physiology

Molecular Dx

Pathology

Pharmacology

Toxicology
Acknowledgements

Bioinformatics
Armin Graber
Olivier Lefèvre
Paolo Zaccaria
Florian Bichteler
Marc Breit
Karl Kugler
Bernd Haas
Mattias Bair
Robert Eller
Hamza Ovacin
Gerd Lorünser
Daniel Andres

Analytics
Steven Ramsay
Sascha Dammeier
Katussevani Bernardo
Therese Koal
Cornelia Röhring
Wolfgang Guggenbichler
Wolfgang Stöggl
Carmen Burgmeier
Ines Unterwurzacher
Peter Enoh
Lisa Körner
Doreen Kirchberg
Verena Forcher

Statistics & Biochemistry
Ingrid Osprian
Marion Beier
Günther Eibl
Gerhard Marini
Matthias Keller
Oliver Lutz
Susanna Olscher

Applied Biosystems / MDS Sciex
Julie Wingate  Karin Wihsböck
Bruno Casetta  Holm Sommer
Joe Anacleto  Gisbert Schäfer
Byron Kieser  Bori Shushan
Dale Patterson  Lyle Burton
Masahide Habu  Chris Elicone
Christoph Kuntzsch  Ron Bonner
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Brad Morie
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Thomas Sieberer

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