Metabonomics in Drug risk Assessment

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From « metabonomic signatures » to biomarker(s) of drugs safety/efficacy
Lead Optimization in Drug Development

Lead Generation

FHD

Phase IA

Phase IB/II

Phase III

Submission

Global Launch

Global Optimization

Target validation

CANDIDATE
Lead optimization acts as a funnel ...

Hundreds of molecules derived from one single chemical platform

- Chemistry (initial low amount synthesis)
- Pharmacology (in vitro efficacy model)
- ADME (metabolism)
- Pre-clinical Toxicology
- Formulation

Only one candidate molecule to enter clinical stages
Toxicological studies are the bottleneck …

- Time and people-consuming
- Request large amounts of compounds
- Need numerous animals (rodents and non-rodents) 3R’s
- Most important target organs to be addressed:
  - Liver
  - Kidney
  - Heart
  - CNS
Toxicological studies are the bottleneck …

➢ Not always predictive of effects seen in humans

Unexplained adverse effects during clinical trials

Idiosyncrasy
withdrawal from the market
Systems biology to speed up L.O. process

Diseases

Chemicals (i.e. drugs)

Gene Mutation

Genomics

Proteomics

Metabonomics

Time

(-)

(+)

(+)

(-)

(-)
Consortium on Metabonomic in Toxicology

Imperial College-London
BMS  Lilly  Novo  Pfizer  Pharmacia  Roche

2001-2004

147 prototoxicants  Acute dosing  \(^1\text{H-}\text{NMR analysis}\)  Database
+ Chemometrics models for toxicity prediction
Experimental Protocol

Metabolism cages on refrigerated racks

Urine

Blood

Pattern recognition

Potential biomarkers

$^1$H NMR
Multivariate MBX data analysis

Binning of the NMR spectra into 250 regions with same spectral width (0.04 ppm)

~ 250 descriptors
Creating an Excel table

Median Chemical Shift of the descriptor 9.86 to 9.90 ppm

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AUC in a descriptor

Each line = $^1$H-NMR spectrum
250 variables or descriptors

10 ppm divided in 250 regions of 0.04 ppm

Binning

Scores

Loadings

Université de Mons
Multivariate MBX data analysis

Scores Plot

3.04 ppm

Creatine

Loadings Plot

3.28 & 3.44 ppm

Taurine
COMET: Control Model

Identifying abnormal urine samples

- 4521 “normal” urine samples
- PCA-based approach
- Determine the distance to the model (DmodXPS+) as well as the probability of belonging to the model (PmodXPS+):

Test sample

- Normal
- Marginal: 95%<x< 99%
- Abnormal: <95%
COMET: CLOUDS Model
(Classification Of Unknowns by Density Superposition)

Identifying toxicity type

- PNN-based model (Probabilistic Neural Network – Specht 1990)

Cardiac toxicity
Renal toxicity
Liver toxicity

Test sample
Clouds approach

Classification based on similarity
From organ-specific toxic signatures to biomarkers identification
Comparative 600 MHz $^1$H-NMR spectra from serum samples from guinea pigs anesthetized with isoflurane (bottom trace) or parenteral pentobarbital containing 40% propylene glycol by volume (top trace). Note the numerous resonances attributable to the propylene glycol in the injected anesthetic.

DRC’s (DRUG RELATED COMPOUNDS)

Acetaminophen and/or metabolites

Urinary excretion of acetaminophen and its metabolites as studied by proton NMR spectroscopy.
Bales JR, Sadler PJ, Nicholson JK, Timbrell JA.
Wrong data interpretation due to bacterial contamination…

Urine collected at 4°C + NaN₃

Bacterial contamination

acétate
lactate
- Animals placed in metabolic cages for urine collection
- Acclimatation (at least 2 days) to minimize stress
- Water and food ad libitum or controlled if necessary

Avoid bacterial contamination!
Biomarker of phospholipidosis

From animals to humans ...

CAD’s (Catationic Amphiphilic Drugs) sequestring phospholipids in vacuoles
reversible effect but often associated with a more severe toxicity

J. Espina et al
Magnetic Resonance in Chemistry (2001); 39(9) : 559 - 565
Excretion of glycine conjugates in urine is species-dependent:

- Benzoic acid converted in hippuric acid in primates, rodents, and rabbits

- Phenylacetic acid is converted in phenylacetylglycine in rats but in phenylacetylglutamine in humans

JK Nicholson et al
Nat rev Drug Discov ; 2002; 1(2) : 153 - 61
Biomarkers of inhibitors of isoprenylation (cancer drugs)

Anti-cancer drug strategy

Inhibition of isoprenylation to prevent binding of small G proteins to cell plasma membranes
Biomarkers of inhibitors of isoprenylation (cancer drugs)

Biomarker of drug efficacy

Dieterle F. et al  
Urine N-acetylfelinine excretion as a biomarker for inhibition of the farnesyl pathway

(Patent of Hoffman-La Roche)
Confounding intrinsic factors

Drug efficacy ⇔ drug safety

For instance, the large reduction in urinary excretion of the TCA cycles intermediates succinate, citrate, and 2-oxoglutarate observed as a result of metabolic acidosis induced by a non toxic renal carbonic anhydrase inhibitor, such as acetazolamide, could possibly be mistaken for a toxic response.

Metabonomics: a platform for studying drug toxicity and gene function
Jeremy K. Nicholson, John Connelly, John C. Lindon & Elaine Holmes
The systemic biochemical effects of oral hydrazine acute dosing have been investigated in male Han Wistar rats using metabonomic analysis of $^1$H NMR spectra of urine.

### Hydrazine liver toxicity

<table>
<thead>
<tr>
<th>Decreased urine excretion</th>
<th>Increased urine excretion</th>
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<tbody>
<tr>
<td>hippurate</td>
<td>taurine</td>
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<tr>
<td>Citrate</td>
<td>Creatine</td>
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<tr>
<td>succinate</td>
<td>threonine</td>
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<tr>
<td>2-oxoglutarate</td>
<td>N-methylnicotinic acid</td>
</tr>
<tr>
<td>TMAO</td>
<td>tyrosine</td>
</tr>
<tr>
<td>fumarate</td>
<td>arginosuccinate</td>
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<tr>
<td>creatinine</td>
<td>N-acetylcitrulline</td>
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**APPEARANCE OF RESONANCES CORRESPONDING TO 2-AMINOADIPATE!!**

**Hydrazine CNS toxicity**

«Mechanistically linked the neurotoxic effects of hydrazine to markedly increased levels of 2-aminoadipate (2AA), which is known to affect kynurenic acid levels in the brain, thus providing a plausible hypothesis for the heretofore unexplained neurotoxic effects of the compound”

Nicholls, A. W., et al  
USUAL SUSPECTS ....

List of urine metabolites which frequently change in response to toxicant administration, regardless of the nature of the toxicant, its mechanism(s) of action, of its target(s). Importantly, not all of these molecules change in response to every toxicant, nor do they necessarily follow the same trajectory (temporal response).

The “Usual Suspects”

2-oxoglutarate
acetate
citrate
creatine
creatinine
glucose
hippurate
lactate
succinate
taurine
trimethyl amine/trimethyl amine oxide (TMA/TMAO)

Robertson DG
Toxicological Sciences 5
(2005) ; 85 : 809-822
The case of hippurate

Drug-induced renal proximal tubular injury

Liver ($\Sigma$)
Kidney (OATP1)
Intestinal flora ($\Sigma$)
Usual suspects … metabolism

Jeeyoun Jung et al, Toxicology Letters 200 (2011) 1–7

Fig. 2. Representative 600-MHz 1H NMR spectra of urine from rats treated with vehicle (A) or naproxen at a low (B), moderate (C), or high (D) dose.
Usual suspects ... metabolism
Conclusions

• Like most technologies, metabonomics will not meet all expectations, but it will certainly add value in many areas of biology

• Significant impact in drug safety assessment

• Metabonomics will be extremely useful in completing the omics circle from gene (genomics) to protein (proteomics) to metabolite (metabonomics)
Conclusions

What will allow determine the realization of this potential?

- Analytical challenges are nearly met (acquisition of data but more by the volume of data and in the complexity of the profiles generated)

- It is in the experimental design and interpretation of metabonomic data that crucial questions remain

Develop biological models from whole organism to cellular and subcellular levels
The team
Thank you for your attention