Use of cellular metabolomics (or fluxomics) for predicting the safety and efficacy of drug candidates

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The Problem; The solution / The offer

➢ The problem

• In pharmaceutical companies, drug development costs over 1 B. US dollars, lasts 10 - 12 years and the attrition rate is high despite more investments.

• Omics (transcriptomics, proteomics, metabolomics)

• Reasons (PK, efficacy, safety)

➢ The offer : Unique approach = cellular metabolomics

• Predict the pharmaco-toxicological interactions of test compounds with the metabolic pathways of any human or animal cell type in vitro

• Contribute to the discovery and development of drug candidates / chemicals (efficacy / safety)
Definitions

Metabolomics = large scale measurement of the metabolite composition of biological fluids (plasma, urine...)

Classical tools

• Magnetic Resonance Spectroscopy
• Mass Spectroscopy

They are complementary
Traditional metabolomics
$^1$H NMR spectrum of normal human urine
\(^1\text{H} \) NMR spectrum of the urine of a patient treated by ifosfamide (IF), an anticancer drug

**before IF**

**after IF**

Metabolic signature:
Glycosuria \(\Rightarrow\) hypothesis

- Is IF diabetogenic? and, if so, by which mechanisms?
- Is IF toxic to the proximal tubule? and, if so, by which mechanisms?
Typical metabolomic study

- **Methods**
  - Very sophisticated analytical techniques

- **Results**
  - Identification of differences by elaborate computations

- **Discussion**
  - “the data suggest that condition X induces substantial alterations in the regulation of pathways Y and Z”

  - This is OK for biomarker identification
  - This is not OK for identifying new mechanisms
Development and applications of cellular metabolomics
Innovative technological approach = Cellular Metabolomics (Metabolic Flux Analysis)

➢ Which combines:

• Metabolically differentiated cell models that retain their in vivo properties

• Measurement of substrate uptake and product formation by enzymatic and complex, innovative techniques (\textsuperscript{13}C NMR)

• Original mathematical models

• A know-how based on a long experience and a skilled multi-disciplinary team

NMR platform € 1.2 M
Cellular Metabolomics (Metabolic Flux Analysis)

➢ Provides in vitro and ex vivo
  • Panoramic view of:
    ✓ Fluxes through cellular metabolic pathways (glucose, glutamine, glutamate, etc...)
    ✓ Adverse/beneficial effects on these pathways

➢ Has been validated and used for
  • Prediction safety/efficacy of cand. / biologics (mg amounts)
  • Metabolic phenotype of cell lines and cancer cells
Effect of antidiabetic drugs or candidates
### Effect of Insulin on the metabolism of $^{13}$C-Glucose in liver cells from fed Wistar rats (incubation for 24 hours) 
(Enzymatic data)

<table>
<thead>
<tr>
<th>Exp. Condition</th>
<th>Glucose</th>
<th>Glycogen</th>
<th>Lactate</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{13}$C-Glucose</td>
<td>-1286 ± 268</td>
<td>-3948 ± 211</td>
<td>1456 ± 125</td>
<td>-7 ± 4</td>
</tr>
<tr>
<td>$^{13}$C-Glucose + Insulin</td>
<td>-3135 ± 305*</td>
<td>-3106 ± 271*</td>
<td>1550 ± 108</td>
<td>80 ± 7*</td>
</tr>
</tbody>
</table>

Values (in µmol/g.protein/24 hrs) are means ± SEM for 6 experiments; *p < 0.05
Effect of Insulin on the metabolism of $^{13}\text{C}\text{-Glucose}$ in liver cells from fed Wistar rats (incubation for 24 hours) (NMR data)

<table>
<thead>
<tr>
<th>Exp. Condition</th>
<th>Glucose (Enz.)</th>
<th>$^{13}\text{C-Glc}$</th>
<th>$^{13}\text{C-Glc}$</th>
<th>$^{13}\text{C-Glg}$</th>
<th>$^{13}\text{C-Lac}$</th>
<th>$^{13}\text{C-TG}$</th>
<th>$^{13}\text{CO}_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{13}\text{C-Glucose}$</td>
<td>-1286 ± 268</td>
<td>-3787 ± 450</td>
<td>851 ± 85</td>
<td>9 ± 2</td>
<td>368 ± 42</td>
<td>77 ± 15</td>
<td>2046 ± 475</td>
</tr>
<tr>
<td>$^{13}\text{C-Glucose}$ + Insulin</td>
<td>-3135 ± 305*</td>
<td>-5981 ± 428*</td>
<td>976 ± 48</td>
<td>148 ± 39*</td>
<td>463 ± 41*</td>
<td>338 ± 52*</td>
<td>3475 ± 447</td>
</tr>
</tbody>
</table>

Values (in µmol/g.protein/24 hrs) are means ± SEM for 6 experiments; *p < 0.05
Acute effect of metformin on the metabolism of $^{13}$C-lactate by liver cells from fed non-diabetic and diabetic ZDF rats
Beneficial and adverse effects of an antidiabetic candidate in rat liver cells

Académie de Pharmacie.
20 February 2013
Valproate efficacy and nephrotoxicity
Valproate efficacy

- Valproate (Depakine) = antiepileptic drug
- MOA: Increase in brain GABA concentration
Effect of valproate on [3-\textsuperscript{13}C]glutamate metabolism (60 min) (NMR data)
Effect of valproate on [3-^{13}C]glutamate metabolism (60 min) (Enzymatic fluxes)

Académie de Pharmacie.
20 February 2013
Valproate nephrotoxicity

Problem: Adverse effect: hyperammonemia

• Inhibition of hepatic urea synthesis
• Stimulation of renal glutamine uptake
• Stimulation of renal ammoniagenesis

Question:
• Which enzymatic step?

Strategy:
• Cellular metabolomics (panoramic view of metabolic pathways)
Effect of valproate on flux through enzymes of glutamine metabolism in human precision-cut kidney-cortex slices

Added value:

- mechanism(s) of toxic effect

- methods of screening for other valproate-related antiepileptics
Effect of nephrotoxic compounds

- Cephaloridine, a β-lactam antibiotic
- Ifosfamide, an anticancer drug
Effect of cephaloridine on the metabolism of $^{13}$C-Succinate in isolated rabbit renal proximal tubules (NMR data)

<table>
<thead>
<tr>
<th>Exp. Condition</th>
<th>Succinate</th>
<th>Fumarate</th>
<th>Malate</th>
<th>Glucose</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C2+C3</td>
<td>C2+C3</td>
<td>C2</td>
<td>C3</td>
<td>C1</td>
</tr>
<tr>
<td>$^{13}$C-Succinate</td>
<td>-14350 ±1159</td>
<td>1816 ±237</td>
<td>3203 ±402</td>
<td>3229 ±404</td>
<td>611 ±39</td>
</tr>
<tr>
<td>$^{13}$C-Succinate + Cephaloridine</td>
<td>-14311 ±1205</td>
<td>2310 ±340*</td>
<td>1428 ±610*</td>
<td>1418 ±634*</td>
<td>258 ±56*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>258 ±43*</td>
</tr>
</tbody>
</table>

Values (in µmol/g dry weight/4 hrs) are means ± SEM for 4 experiments; *p < 0.05
Effect of cephaloridinidine on the production of $^{13}\text{CO}_2$ from $^{13}\text{C}$-Succinate and on the ATP cellular levels

<table>
<thead>
<tr>
<th>Exp. Condition</th>
<th>$^{13}\text{CO}_2$ From [1,4-$^{13}\text{C}$]succinate</th>
<th>$^{13}\text{CO}_2$ From [2,3-$^{13}\text{C}$]succinate</th>
<th>ATP (µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{13}\text{C}$-Succinate</td>
<td>4887 ±680</td>
<td>290 ±51</td>
<td>7.28 ±0.96</td>
</tr>
<tr>
<td>$^{13}\text{C}$-Succinate + Cephaloridinidine</td>
<td>2966 ±377*</td>
<td>342 ±14</td>
<td>4.97 ±0.48*</td>
</tr>
</tbody>
</table>

Values (in µmol/g dry weight/4 hrs) are means ± SEM for 4 experiments; *p < 0.05
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Glucose → Pyruvate → Lactate → (Chloro)acetaldehyde

Pyruvate → Oxaloacetate → Acetyl-CoA → (Chloro)acetate

Acetyl-CoA → Krebs Cycle → 2CO₂

LDH: Lactate Dehydrogenase
PC: Pyruvate Carboxylase
PDH: Pyruvate Dehydrogenase
CoA: Coenzyme A
Acute toxicity of CAA

Human kidney tubules incubated for 1 hr

% of LDH released

Concentration of CAA (mM)

CAA (mM)

0 0.1 0.2 0.3 0.4 0.5

µmol/g dry wt

ATP

Concentration of CAA (mM)

0 0.1 0.2 0.3 0.4 0.5

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Metabolism of 2-\textsuperscript{13}C-chloroacetaldehyde by human renal proximal tubules (\textsuperscript{13}C NMR spectra)

\begin{itemize}
\item \textbf{T=0 min}
  \begin{itemize}
  \item 48.5 ppm CAA surf: 19408
  \item 42.7 ppm GLY surf: 26375
  \end{itemize}
\end{itemize}

\begin{itemize}
\item \textbf{T=60 min}
  \begin{itemize}
  \item 44.9 ppm CAT surf: 9476
  \item 42.7 ppm GLY surf: 15188
  \end{itemize}
\end{itemize}

\textsuperscript{13}C NMR spectra

\textsuperscript{13}C NMR spectra
Cellular metabolomics:

- Provides in vitro a panoramic view of the metabolism of physiological substrates in any normal or pathological human or animal cell type.
- Combines enzymatic, (radioactive) and $^{13}$C NMR methods with mathematical models of metabolic pathways.
- Allows to identify and quantify (fluxes) metabolic pathways.
- Is complementary of but differs from other omics in that it provides functional pieces of information.
• Allows to identify early in the drug development process the pharmaco-toxicological interactions of very small amounts of drug candidates (acute and chronic effects).

• Therefore allows to assess simultaneously the efficacy and safety of drug candidates:
  ✓ Efficacy (areas: metabolic diseases; oncology; neurodegenerative diseases)
  ✓ Safety: any drug from any therapeutic area
Glutamine metabolism in kidney tubules from fed and fasted rats
General scheme of glutamine metabolism in mammalian tissues

Glucose → Phosphoenolpyruvate → Pyruvate → Lactate

Glucose → CO₂ → Oxaloacetate → CO₂ → CO₂ → Citrate → Acetyl-CoA

Aspartate → CO₂ → Oxaloacetate → CO₂

α-Ketoglutarate → CO₂ → Glutamate → NH₄⁺ → GLDH → Aspartate

Glutamate → NH₄⁺ → Glutamine → GLNase → GSH → GABA

Puric and pyrimidic bases → Pyruvate → Lactate

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$^{13}$C NMR spectra (125.75 MHz) of neutralized perchloric extracts obtained from fed (A) and fasted (B) rat kidney tubules incubated with [3-$^{13}$C] glutamine.

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Labeling of the C1, C2, C5 and C6 of glucose, and the C2 and C3 of lactate and alanine from [3-^{13}C]glutamine

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Schematic representation of the fate of the C1, C2 and C3 of glutamine in rat kidney tubules

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Effect of fasting on fluxes through pathways of glutamine metabolism in rat kidney tubules

\[ \text{Glucose} \rightarrow \text{Pyruvate} \rightarrow \text{Oxaloacetate} \rightarrow \text{DONCE cycle} \rightarrow \text{Glutamate} \rightarrow \text{Glutamine} \]

- Multicycle
- Complexity of gluconeogenesis
- Heterogeneity

\( n = 4 \) experiments performed in quadruplicate; \( \mu \text{mol.g dry wt}^{-1.\text{h}^{-1}} \); * \( P < 0.05 \)