Metabonomic evaluation of hepatotoxicity induced by chemical substances in *in vivo* and *in vitro* models.

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Introduction

Metabolomics studies from urine analysis is a new useful tool to evaluate drug-induced toxicities[1]. However, due to the multiorganic origins of urine components, it is often difficult to determine which organ/tissue a particular metabolite is arising from. Therefore, we are going to use the isolated and perfused rat liver combined to the analysis of perfusion media by HR ^{1}H-NMR. The comparison of the effects of hepatotoxic compounds (acetaminophen, hydrazine and methapyrilene) in *in vivo* and *in vitro* should allow us to unambiguously identify biomarkers of hepatotoxicity.

Material and method

Isolated and perfused liver model : male Wistar rats were anaesthetized with isoflurane. After laparotomy, the portal vein was catheterized for perfusion with Krebs-Henseleit buffer via a peristaltic pump. Toxins were added directly in the perfusion liquid at the same concentration used *in vivo*. The liquid of perfusion was sampled every 10 minutes and prepared for ^{1}H NMR spectroscopy. Acid extractions of liver were also carried out.

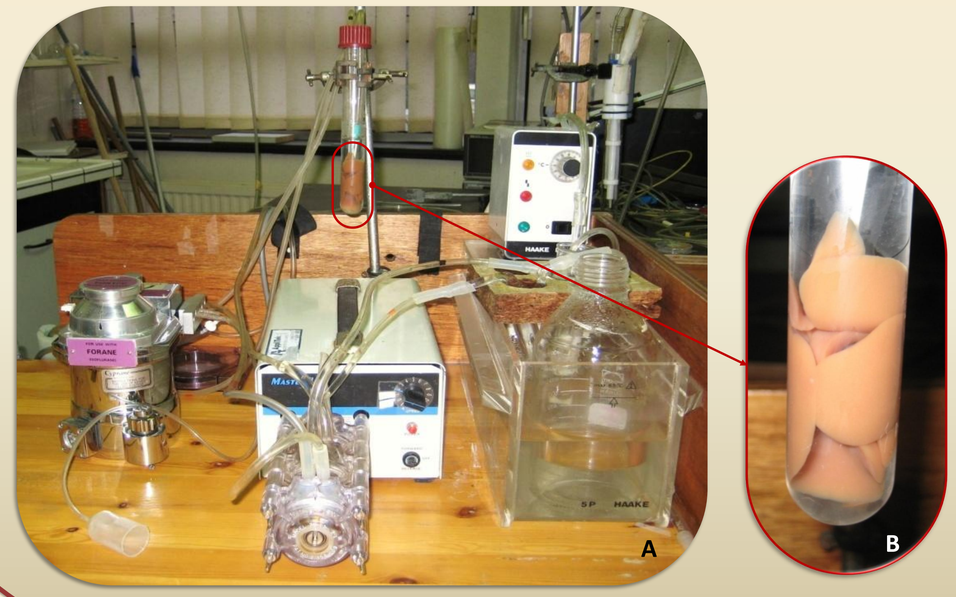
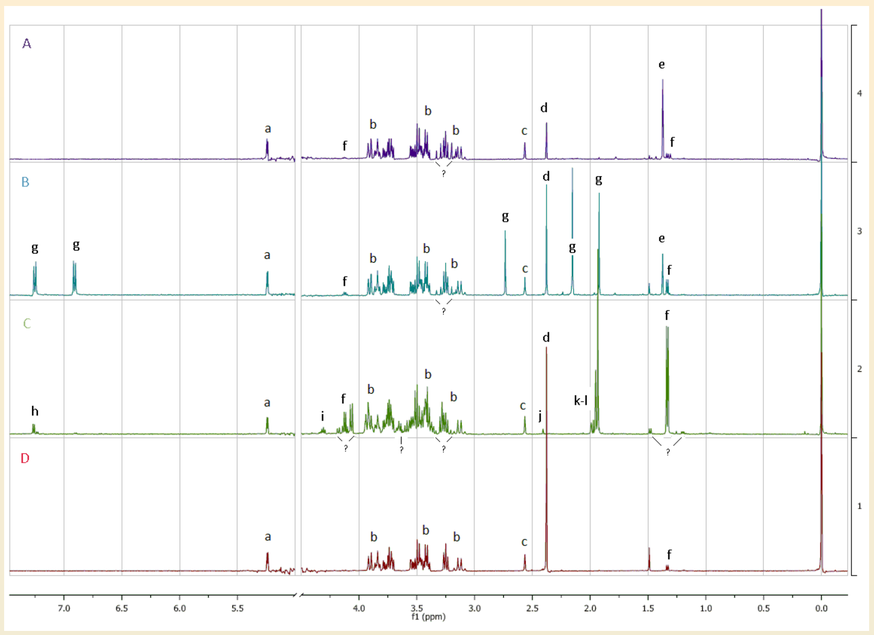


Figure 1. **A**: Picture of perfusion equipment with peristaltic pump. **B**: Soak liver.

Results



a : α glucose
b : sugar
c : EDTA ?
d : pyruvate
e : α hydroxyisobutyrate
f : lactate
g : APAP
h : hydrazine
i : thréonine ?
j : succinate
k-l : diacetylhydrazine
acetylhydrazine

Figure 2. **^{1}H NMR spectra, at 500MHz,** of perfusion media
A : from control liver
B : from liver treated with acetaminophen
C : from liver treated with hydrazine
D : from liver treated with methapyrilene

These spectra are preliminary results.
The combined increase in creatine and taurine after the treatment with hydrazine is associated with liver necrosis [2] and elevation in lactate is correlated with the decrease of pyruvate.
In the other spectra, modifications are due to resonances arising from drug related compounds.

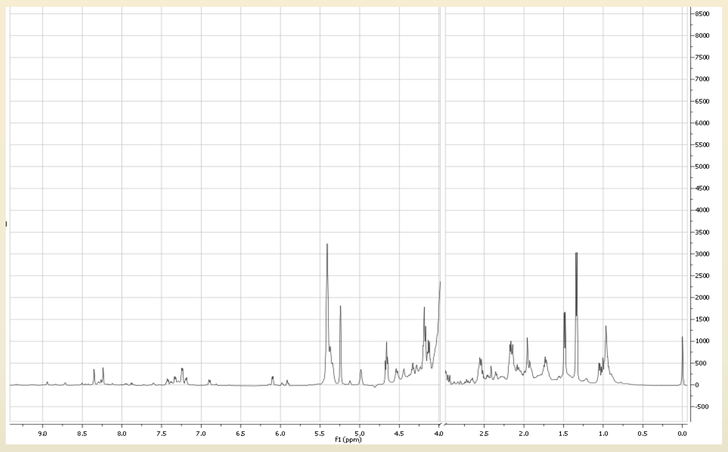


Figure 3. **^{1}H NMR spectra, at 500 MHz,** of acide extraction of liver treated with hydrazine.

^{31}P NMR spectra of liver soak in the perfusion media was used to check the good health of liver cells before toxin addition. The ratio between ATP and inorganic phosphorus is used as a marker. The substances are added at sublethal dose.

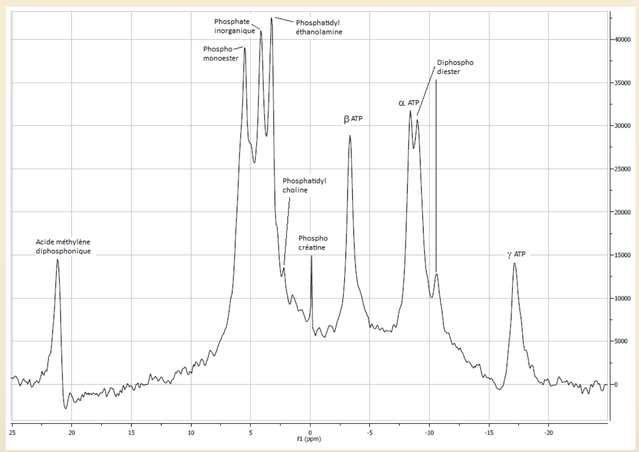


Figure 4. **^{31}P NMR spectra, at 300 Mhz,** of liver soak in the perfusion liquid.

Conclusion and perspective

Each toxin induces a different metabolic fingerprinting which is characteristic of a type of hepatotoxicity.
Isolated and perfused liver is an intermediate model between in vivo and primary hepatocyte in culture. Its greater advantage is to determine the biomarkers of toxicity which come exclusively from the liver. Our ultimate goal is to use cellular cultures to screen new chemicals and to investigate the cellular mechanism of toxicity.

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Reference :
[1]: *Lindon J.C. & al*. Contemporary issues in toxicology The role of metabonomics in toxicology and its evaluation by the COMET project. Toxicol & applied pharmaco , 187 (2003) 137-146
[2] : *Bollard M.E. & al*. Comparative metabonomics of differential hydrazine toxicity in the rat and mouse. Toxicol & applied pharmaco, 204 (2005) 135-151
[3] : Sun J & al. Metabonomics evaluation of urine from rats given acute and chronic doses of acetaminophen using NMR and UPLC/MS. Journal of chromato B, 871 (2008) 328-340