METABONOMIC FINGERPRINT OF THE ISOLATED AND PERFUSED LIVER.

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Background :

Metabonomics studies based on spectroscopic analysis of biofluids are a useful tool to explore the biological and metabolic profiles of an organism. However, biofluids are a mixture of numerous metabolites with various tissue origins, making it very difficult to determine which organ/tissue a particular metabolite is arising from (fig.1).
This is particularly troublesome when attempting to validate urinary markers of hepatotoxicity because most of them could as well be excreted by extra hepatic cells. In order to overcome this issue, we applied the metabonomic approach to the **isolated and perfused rat liver** (IPRL).

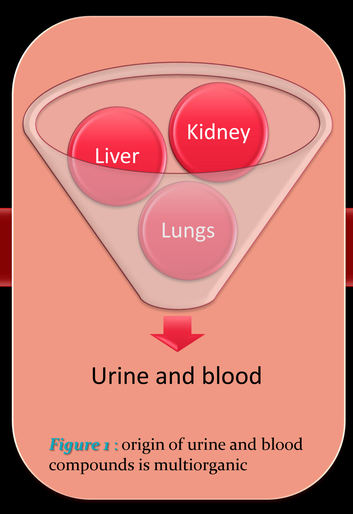


Figure 1 : origin of urine and blood compounds is multiorganic

The IPRL is a valuable ***ex vivo* model** commonly used in metabolic, transport, PK/PD, and toxicology evaluation of xenobiotics.
Indeed, the liver tissue can be exposed to the test substance independently from other organs. The perfusion fluid can then be sampled over time and subsequently analyzed by spectroscopic techniques (NMR or SM) for metabolic as well as risk assessment purposes.
Finally, as compared to cultured hepatocytes, the IPRL model maintains the tissue architecture, cell polarity and bile flow.

Applying the metabonomic approach to the IPRL model should allow us to undoubtfully **identify** which markers are indeed of hepatic origin.

Material and methods :

Livers isolated (fig.2a) from male Wistar rats were perfused through the portal vein with 250ml of a recycling Krebs-Henseleit solution using a peristaltic pump (fig.2b).

Perfusion fluid was sampled every 15 minutes for two hours. Samples were prepared for ^{1}H-NMR spectroscopy by adding 200µl of phosphate buffer (with D_2O) to 400µl of fluid. TSP was used as external reference. At the end of the experiment, the entire volume of recirculating perfusion liquid was lyophilyzed for NMR analysis. During the entire experiment, liver viability was assessed by ^{31}P NMR. Liver resections were prepared either for histopathology review or for NMR analysis after acid extraction.

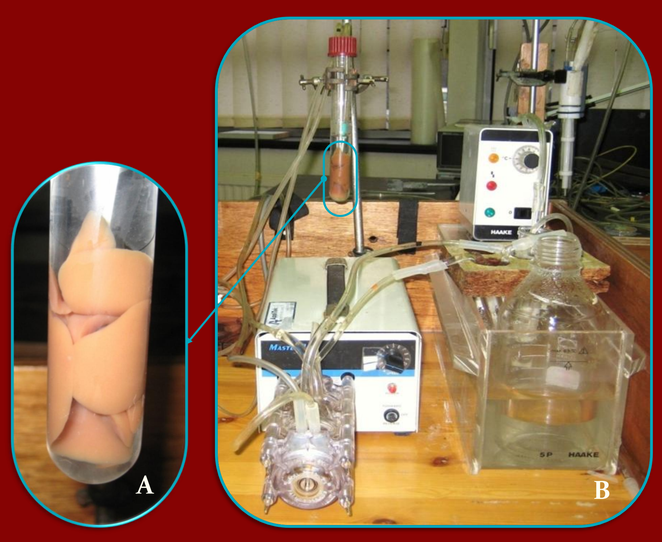


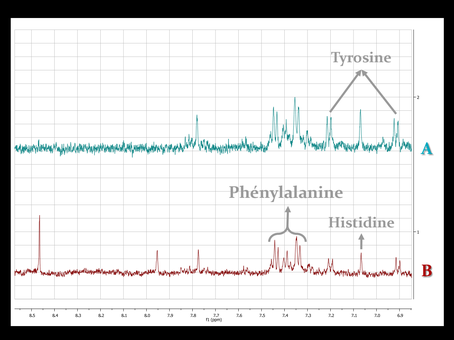
Figure 2. **A**: Isolated liver. **B**: Picture of perfusion equipment with peristaltic pump.

Results :

The metabonomic fingerprint obtained by ^{1}H-NMR spectroscopy of viable perfused livers (fig.3; spectrum A) fairly well correlated with high ATP levels as measured by ^{31}P-NMR (ATP/Pi > 1) (fig.4a).
In opposite, livers in unhealthy energetic status (ATP/Pi < 1) (fig.3; spectrum B) presented signs of metabolic alterations, mainly seen as large decreases in amino acids (Aas), glycogen stock (data not shown), ascorbate (an antioxidant) and glucose.
The highest levels of hepatic enzymes (AST and ALT) (table 1) released in the perfusion fluid of unhealthy livers confirmed those metabolic observations and perfectly matched the decreases in ATP contents (fig.4b). Finally, when healthy livers were exposed to an hepatotoxicant added to the perfusion fluid, specific metabolic changes were also observed as compared to pretreatment fingerprint.

	Healthy	Unhealthy
ALT	255 IU/L	710 IU/L
AST	133 IU/L	447 IU/L

Table 1 : **levels of hepatic enzymes.**
ALT: alanineaminotransferase
AST: aspartateaminotransferase



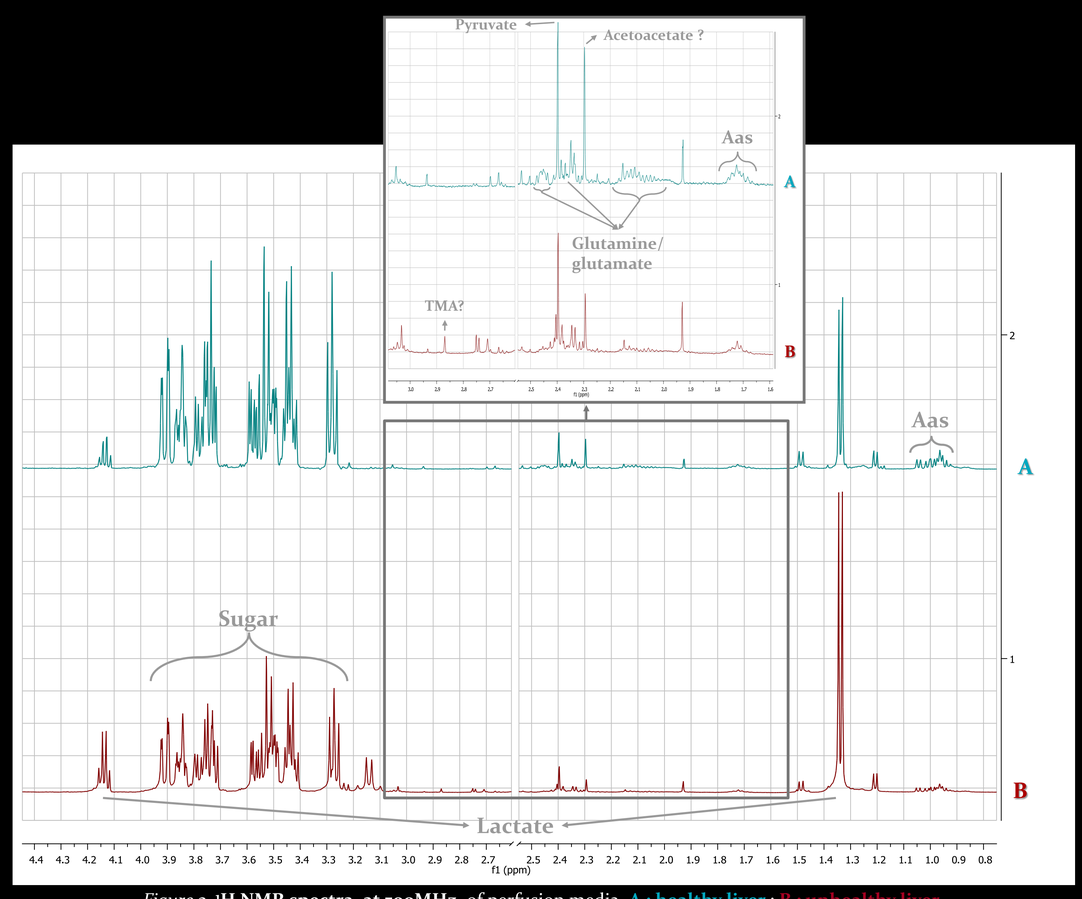
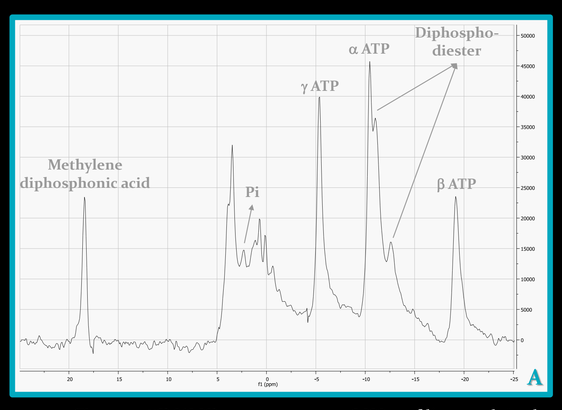


Figure 3. **^{1}H NMR spectra, at 500MHz,** of perfusion media. **A : healthy liver** ; **B : unhealthy liver**
TMA : trimethylamine



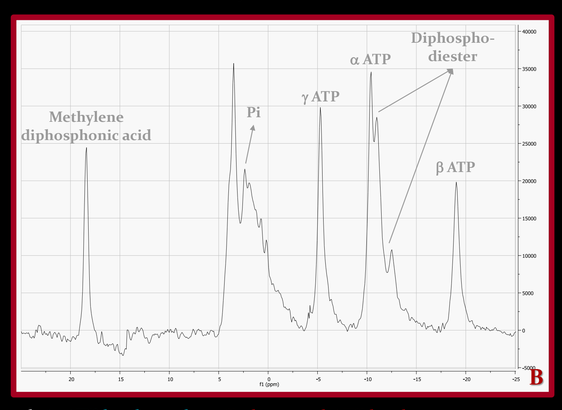


Figure 4. **^{31}P NMR spectra, at 300 MHz,** of liver soak in the perfusion liquid. A: ATP level standart ; B: decreased ATP level

Perspectives:

Discovery of new biomarkers of drug-induced toxicity is a promising application of metabonomics. However, urinary or plasma components may originate from many different tissue/organs which makes it very difficult to validate such markers. The isolated and perfused organ approach could be a very useful tool to **confirm the tissue origin of proposed biomarkers before their validation**.