Evaluation of Idiosyncratic Hepatotoxicity in rats by a metabonomic approach

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Introduction

Idiosyncratic drug reactions (IDR's) occur in small subset of patients and are unrelated to the pharmacological action of the drug(1). They are unpredictable and rare, but with a fatal outcome. IDR's of a hepatic origin are a major health concern. The idiosyncratic toxicities are not driven only by drug exposure but rather depend on several drug- and patient-related risk factors(2). Beside the well known immunological contribution, reactive metabolites are believed to be responsible for most idiosyncratic drug reactions, although no undeniable evidence has been provided so far(3).

This study aims at developing an animal model to assess the metabolic contribution of IDR's. The metabolic mechanism of idiosyncratic reaction is investigated using a metabonomic approach. Some drugs known to produce idiosyncratic reactions in humans are evaluated on the animal model. This poster presents preliminary data recently obtained.

Materials and Methods

In these experiments, three Wistar Han rats by treatment group were individually placed in metabolism cages. They were allowed free access to water and received 30 to 35 gr of food/day. In this study, two models were developed. In the "metabolic model", rats were given 2.5 x 10^6 EU/Kg lipopolysaccharide (LPS) (*Escherichia coli* serotype O55:B5) or its vehicle, i.p.. 2 hours after this treatment, a sub toxic dose of a drug known to produce idiosyncratic reaction in humans or its vehicle was administered i.p. or i.v.. In the "immunologic model"; rats received daily a sub toxic dose of a drug known to produce IDR's in humans or vehicle. From Day -2 to Day 3, fractions of urine were collected in tubes containing sodium azide (solution 1%). Urine was collected once a week during 34 days of experiment. Then the samples were analysed by NMR on an Avance 500 Bruker (11.8T) at 500 MHz for ^{1}H observation. 8 hours after the injection of LPS, a sampling of blood was made on animals to quantify the TNF-alpha using Rat TNF-alpha ELISA test to evaluate LPS-mediated inflammation.

Dosage of TNF-alpha

Rat traited with LPS
TNF-alpha = 291.78 ± 115.60 pg/ml

Rat traited with vehicle
TNF-alpha = 30.54 ± 43,19 pg/ml

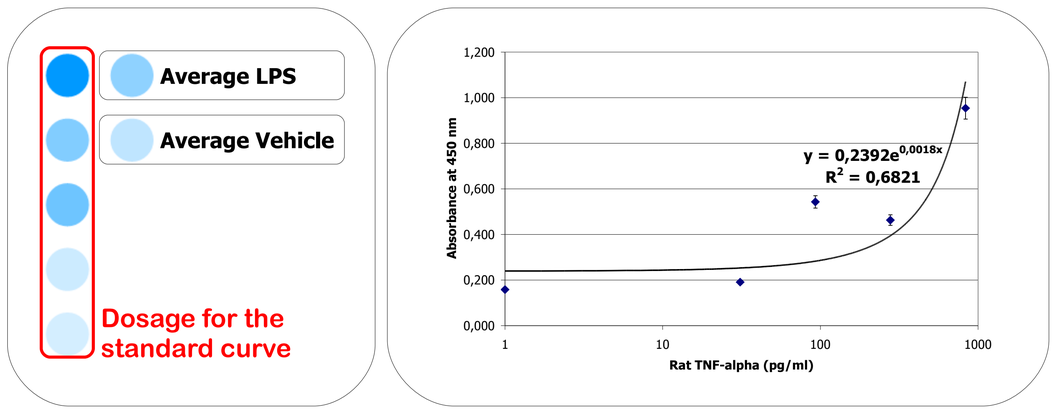

Fig.1: A-Representation of the ELISA test ; B-Plot of the Standard curve, absorbance obtained for each Standard concentration on the vertical (Y) axis vs. the corresponding TNF-alpha concentration (pg/ml) on the horizontal (X) axis.

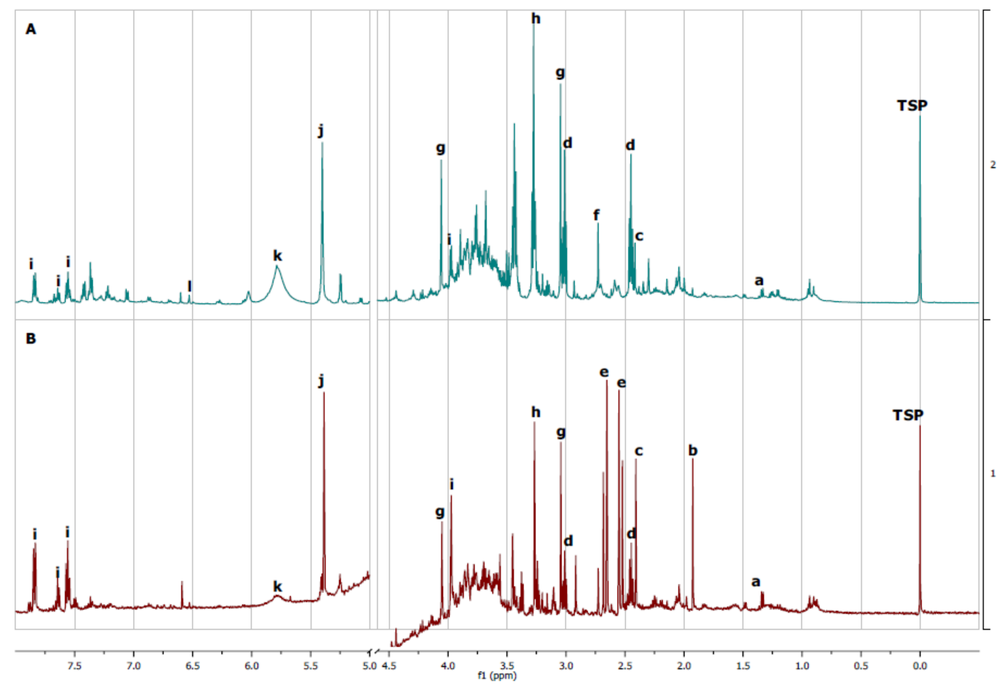

Fig.2: ^{1}H NMR spectra of urine (20 to 24 h), comparison LPS or Vehicle treatment. Rats were treated with 2.5 x 10^6 EU/kg LPS (A) or vehicle (B). Peaks showing major differences and the components identified: (a) lactate, (b) acetate, (c) succinate, (d) alpha-ketoglutarate, (e) citrate, (f) DMA, (g) creatine/creatinine, (h) TMAO, (i) hippurate, (j) allantoin, (k) urea and (l) fumarate.

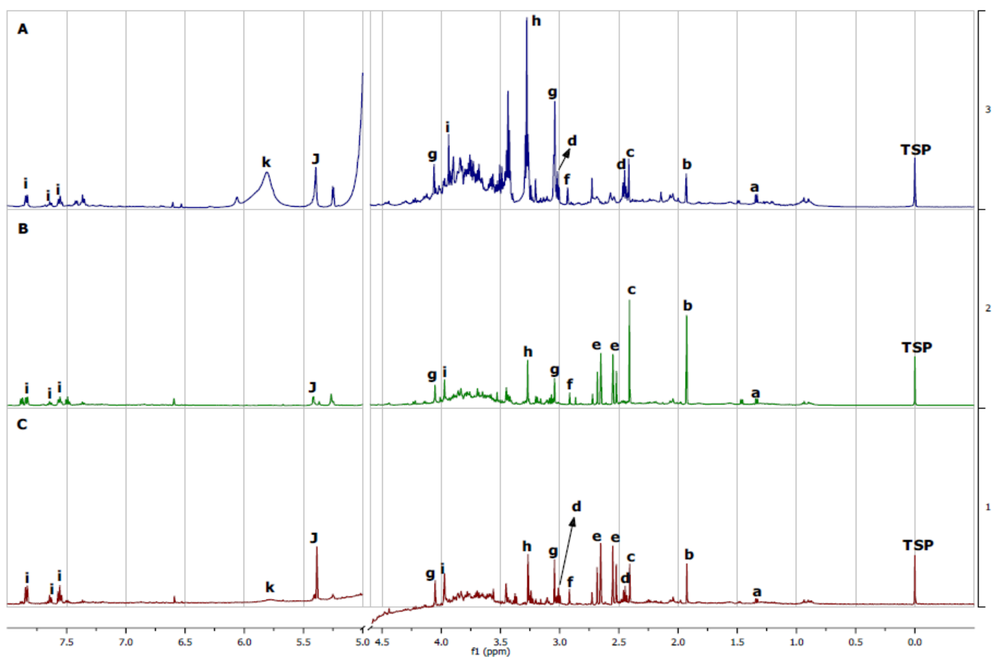

Fig.3: ^{1}H NMR spectra of urine (20 to 24 h) for "metabolic model". Rats were pretreated with 2.5 x 10^6 EU/kg LPS and after 30 mg/Kg Ranitidine (A) or Rats were pretreated with Vehicle and after 30 mg/Kg Ranitidine (B) or Vehicle (C). Peaks showing major differences and the components identified: (a) lactate, (b) acetate, (c) succinate, (d) alpha-ketoglutarate, (e) citrate, (f) DMA, (g) creatine/creatinine, (h) TMAO, (i) hippurate, (j) allantoin, (k) urea and (l) fumarate.

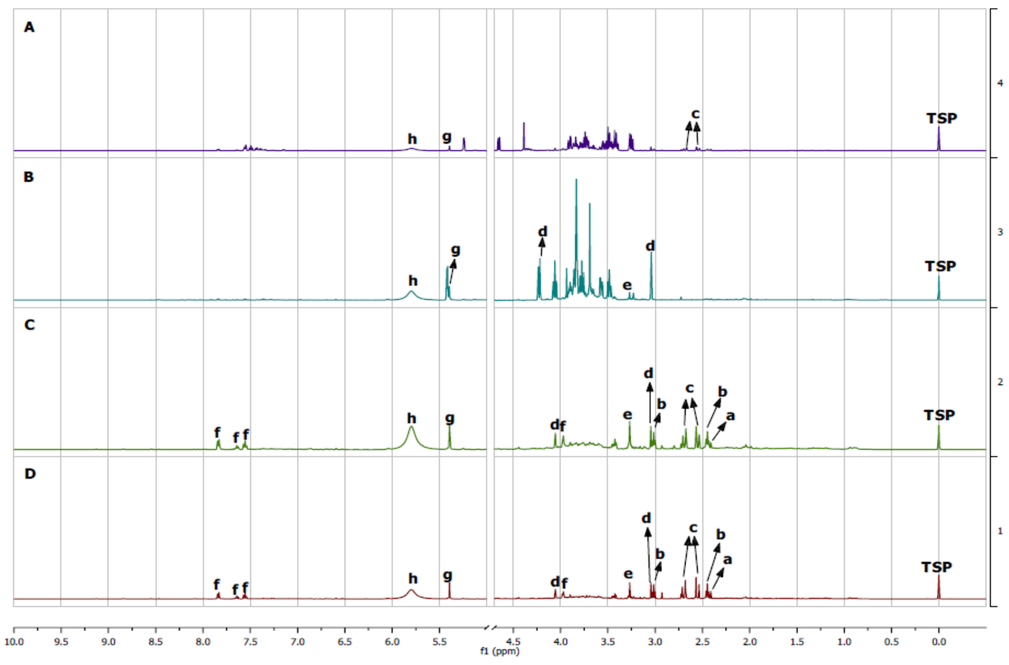

Fig.4: ^{1}H NMR spectra of urine (456 to 480 h) for "immunologic model". Rats were treated with 800 mg/Kg Felbamate (A), 100 mg/Kg Nevirapin (B), 30 mg/Kg Ranitidine (C) or Vehicle (D). Peaks showing major differences and the components identified: (a) succinate, (b) alpha-ketoglutarate, (c) citrate, (d) creatine/creatinine, (e) TMAO, (f) hippurate, (g) allantoin and (h) urea.

Table1: Changes in endogenous urinary metabolites 20 to 24 h after LPS/RAN treatment.

Endogenous metabolite Increase	Endogenous metabolite Decrease
TMAO	Hippurate
alpha-glucose	Allantoin
Lactate	Citrate
Creatine/Creatinine	

Discussion and Conclusion

In this preliminary study, LPS pre-treatment increased the blood TNF-alpha level (Fig 1) and modified the urine metabolic composition. These changes included increases in alpha-ketoglutarate and phenylalanine as well as decrease in citrate (Fig 2).
This study also indicated that Idiosyncrasy-like injury develops in "Wistar Han" rats when they are cotreated with LPS and Ranitidine (RAN). The urine from cotreated rats showed metabolic changes that were not seen in urine from rat treated with RAN alone (Fig 3). Urinary metabolic changes in LPS/RAN-traited rats included increases in creatine & creatinine and decrease in citrate (Table 1). These changes have been associated with hepatotoxicity.
The important changes in the urinary composition of rats treated with Felbamate or Nevirapin are probably an immunological reaction induced by these drugs. It remains however to establish a link between this reaction and the modified metabolites.

Acknowledges

NMR measurements were performed in the Departement of General, Organic, and Biomedical Chemistry of U Mons. We are grateful to Dr L. Vander Elst and Dr S. Laurent for their support in NMR spectra acquisition.

Référence

(1) Shaw PJ, Hopfensperger MJ, Ganey PE, Roth RA ; Lipopolysaccharide and trovafloxacin coexposure in mice causes idiosyncrasy-like liver injury dependent on tumor necrosis factor-alpha ; Toxicol Sci. 2007 Nov ; 100(1):259-66.

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