^{1}H-NMR based metabonomics as a potential tool to assess cardiotoxicity in drug development

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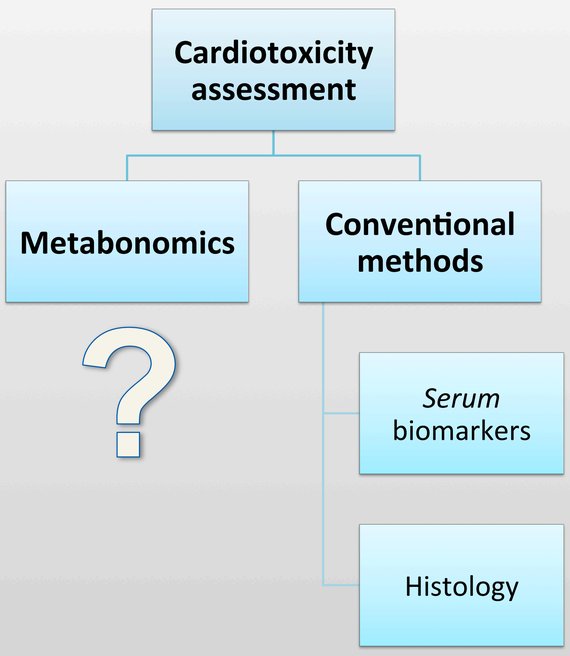
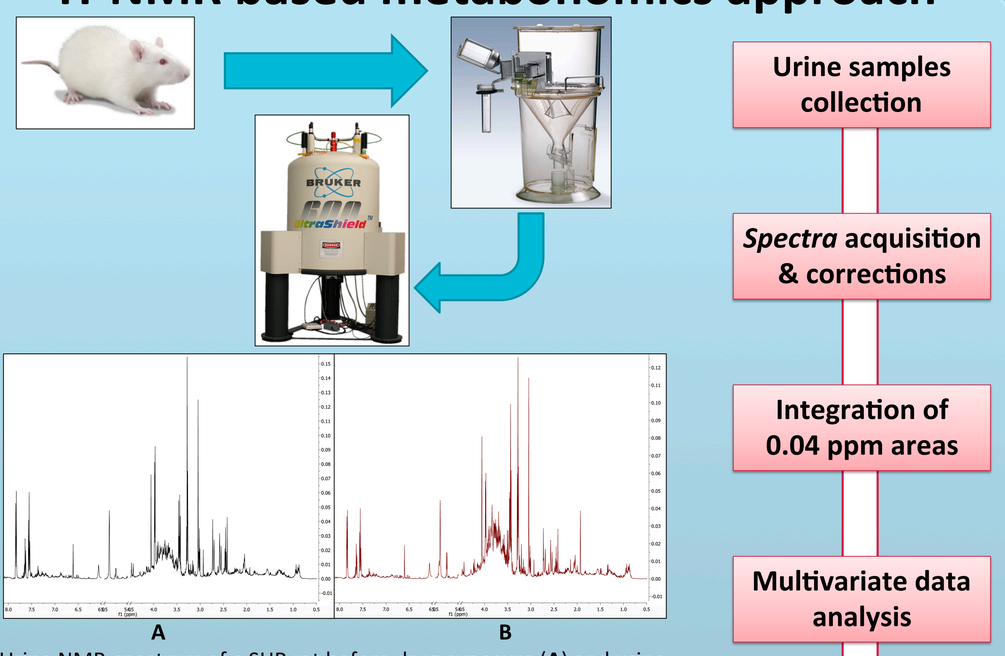
Currently, the main cause of drug post-approval withdrawal from the market is cardiovascular unexpected events. Indeed, safety cardiovascular issues account for 45% of the total post-approval withdrawal, compared to 32% for hepatic issues *(1)*. The severity and the high incidence of cardiovascular toxicity in the late-stage of clinical drug development can lead to several consequences : restrictions of medical use, special pre- and/or post-approval monitoring and drug withdrawal. However, cardiovascular toxicity accounts only for 9% of drug attrition during phase I of clinical development *(2)*. The data suggest that there is a need to develop more predictive methods to assess cardiotoxicity in preclinical studies and in early stages of clinical trials to avoid the progression of drug candidates with a high risk for the cardiovascular system. Therefore, a ^{1}H-NMR based metabonomics was used to reassess the cardiotoxicity of 2 nonsteroidal anti-inflammatory drugs (NSAIDs), Diclofenac and Rofecoxib, recently incriminated with an increased incidence of heart infarct *(3,4)*.

Material & method

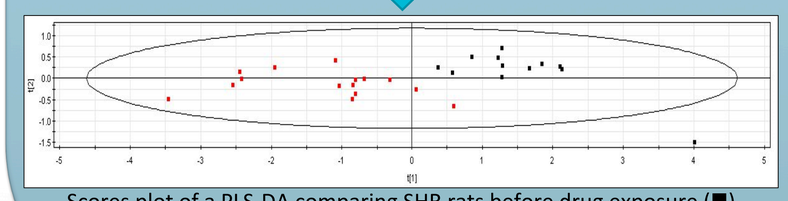
Diclofenac and Rofecoxib were selected for our investigations. The SHR rat strain was used as a hypertension model in view to potentiate the NSAIDs cardiotoxicity. Rats were exposed *per os* daily to the one or the other molecules during a 28 days treatment and were randomly divided into 3 groups for each study : a control group (n=3), a low dose group (n=5) and a high dose group (n=5). Blood samples were collected during the treatment for protein biomarkers dosage. The rats were euthanized at different times of the studies for cardiac histology. For each study, a ^{1}H-NMR based metabonomics analysis was carried out on urine samples, using a 500.16 MHz Bruker Avance spectrometer with a 5 mm DUX 3H-1H probe. A multivariate data analysis was performed to highlight discriminant metabolites. The data obtained were compared to histology and *serum* conventional biomarkers.



^{1}H-NMR based metabonomics approach



Urine NMR *spectrum* of a SHR rat before drug exposure (**A**) and urine NMR *spectrum* of a SHR rat exposed to Diclofenac Sodium at week 1 (**B**).

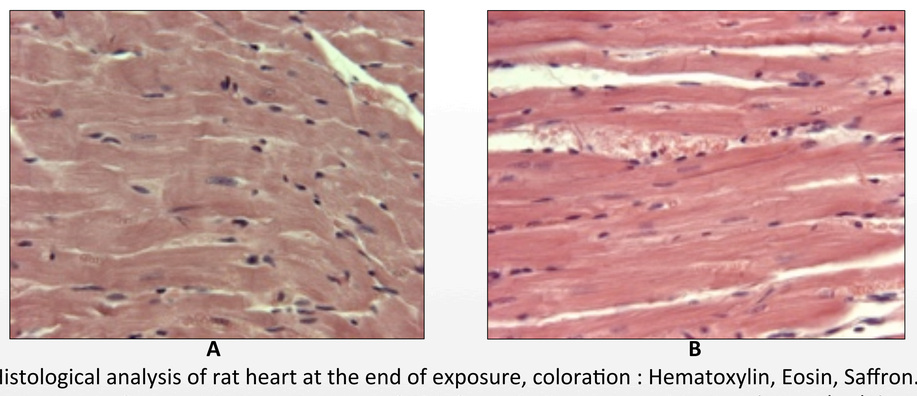


Scores plot of a PLS-DA comparing SHR rats before drug exposure (■) and SHR rats exposed to Diclofenac Sodium at week 1 (10 mg/Kg/D) (■). Model parameters : $R^2cum = 0.77$; $Q^2cum = 0.6$; $p < 0.01$ (CV-ANOVA).

Results : Conventional methods

Histology

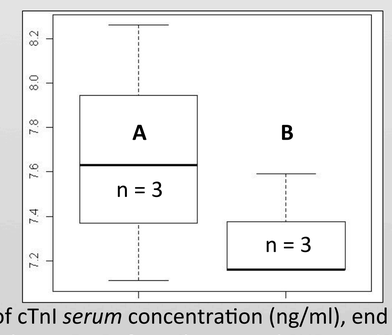
Heart histological analysis were performed in control and exposed rats at the end of the study. Some exposed rats were also sacrificed after 2 and 3 weeks of exposure for cardiac histological monitoring. Histology shows no morphological difference between control and exposed rats.



Histological analysis of rat heart at the end of exposure, coloration : Hematoxylin, Eosin, Saffron.
A : Heart of control SHR rat; **B** : Heart of Diclofenac Sodium exposed SHR rat (10 mg/Kg/D).

Protein biomarkers measurement

Cardiac Troponin I (cTnI), cardiac myosin light chain 1 (CMLC-1) and myoglobin *serum* levels were measured by ELISA assays. No significant difference was found between control and exposed rats.



Box plot of cTnI *serum* concentration (ng/ml), end of exposure.
A : Control rats; **B** : Rofecoxib exposed SHR rats (30 mg/Kg/D).

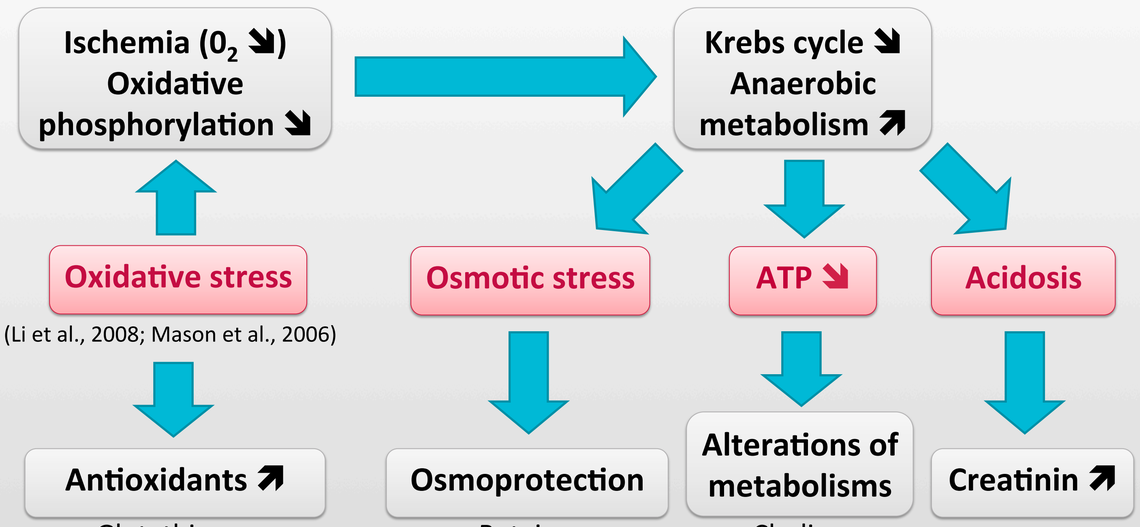
Results : Metabonomics

Multivariate data analysis were performed to highlight changes (VIP value > 0.8) in discriminant metabolites. Main metabolite changes are shown in the table below (arrows indicate the direction of changes for the drug-exposed groups, compared to control group).

Identified metabolites	Diclofenac exposed rats			Rofecoxib exposed rats		
	Week 1	Week 2	Week 4	Week 1	Week 2	Week 4
α-Ketoglutarate	↘	↗	↘	↘	↗	↘
Allantoin	↘	↗	↗	↘	↗	↘
Betain	↗	↘	↘	=	↘	↗
Citrate	↘	↗	↘	↘	↗	↘
Creatinin	↘	↗	↗	=	↗	↘
Dimethylamine (DMA)	↘	↘	↘	=	=	↘
Dimethylglycine (DMG)	=	=	↘	=	=	=
Hippurate	↘	↗	↘	↘	↗	↘
Methylamine	=	↗	=	=	=	=
Succinate	↘	↗	↘	↘	↗	↘
Taurine	↗	↘	↘	↗	↘	↘
Trans-Aconitate	=	=	↘	↗	=	=
Trimethylamine N-oxide (TMAO)	↗	↘	↘	=	=	↘

Discussion & conclusion

Cardiotoxicity of Diclofenac and Rofecoxib was not demonstrated by conventional methods (histology and *serum* biomarkers) in SHR rats. Our ^{1}H-NMR based metabonomics approach highlighted discriminant metabolites in collected urine samples of exposed rats. These changes allowed us to infer into biochemical pathways alterations related to early ischemia stages of heart :



References

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2. Sibille M, Deigat N, Janin A, et al. Adverse events in phase-I studies: a report in 1015 healthy volunteers. Eur J Clin Pharmacol. 1998 Mar;54(1):13–20.
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