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In vivo and *in vitro* NMR-based metabolomics for studying clioquinol treatment effects on brain metabolism in an Alzheimer's disease mouse model

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Introduction

Alzheimer disease (AD) is characterized by amyloïd deposits in brain tissues and neurofibrillar degenerations closely associated with a local inflammatory process, triggering neuronal death. The main constituent of amyloid deposits is a peptide called amyloid beta (Aβ). Nowadays, there is neither curative medicine, nor effective and valid animal model for the evaluation of new medicines against AD. It could thus be important to discover efficient diagnostic tools allowing to reveal the disease before the first signs of cognitive decline and to evaluate the effects of drugs.

Metabolomic offers a global approach for describing metabolic changes in a cell, tissue or organism. The analysis of the metabolome and its variations is a powerful tool to reveal the answer of living systems to endogenous or exogenous disturbances. Metabolomic analysis consists in identification and as much as possible quantitation of the largest set of metabolites. **The goal of this study was to compare the metabolomes of hippocampus from clioquinol-treated and untreated control and AD mice obtained with *in vivo* and *in vitro* NMR.**

Experimental part

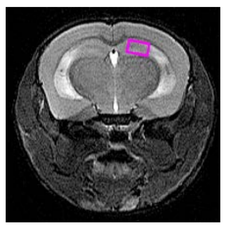
➢ Animals and treatment

3 month-old AppSwe Tg2576 control (Tg2576-) or transgenic (Tg2576+) mice were used in this study [1]. Hippocampus were analyzed with MRS after two months (between 1 and 3 months) of treatment with a solution of clioquinol or with the vehicle alone. After MRS, animals were sacrificed by cervical dislocation. Hippocampus was removed, immediately frozen in liquid nitrogen and then stored at -80° until extraction.

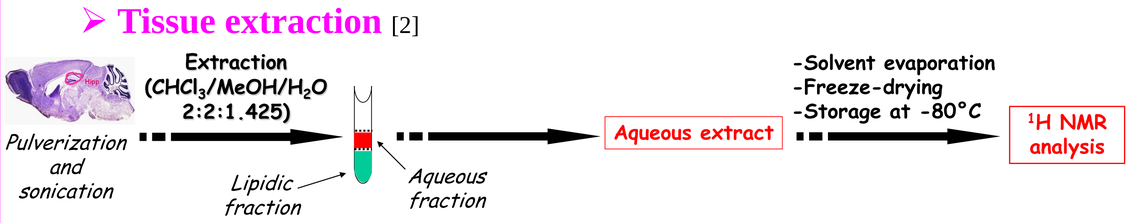
Number of mice	Vehicle	Clioquinol
Control	7	8
Transgenic	6	6

➢ MRI and MRS

Mice were anesthetized with 1.5% isoflurane. They were placed in animal handling system. The physiological parameters (temperature, respiratory frequency) were monitored. The mouse head was centered in birdcage coil. All experiments were performed on a Avance II 400WB. The VOI was positioned in the hippocampus and the voxel size was 1.27mm^3. A PRESS sequence (TE: 8.8 ms; TR: 4s; NS: 1024) was used. Metabolite concentrations were determined by QUEST (jMRUI®).



➢ Tissue extraction [2]



➢ NMR

The 1D ^{1}H NMR experiments were recorded at 298K on a Bruker Avance 400 spectrometer equipped with a 5 mm TBO probe. 1D NMR sequence (relaxation delay-pulse-acquisition) including water presaturation was used. Acquisition parameters were 4.1 s and 2 s for acquisition time and relaxation delay respectively, with a spectral width of 10 ppm and a flip angle of 30°.

➢ Statistical analyses

Bin area method based on intelligent bucketing (KnowItAll®, BIORAD) was used for segmenting RMN spectra. The integrated regions were normalized and data preprocessed by mean-centering. Univariate (ANOVA, BoxPlot) and multivariate approaches (PCA, PLS-DA) were applied to *in vivo* and *in vitro* data for metabolite discrimination using the free R software and SIMCA P+ (Umetrics®). The parameter Q^2 (predictive value of the model) was used to validate each model.

NMR and MRS assignments

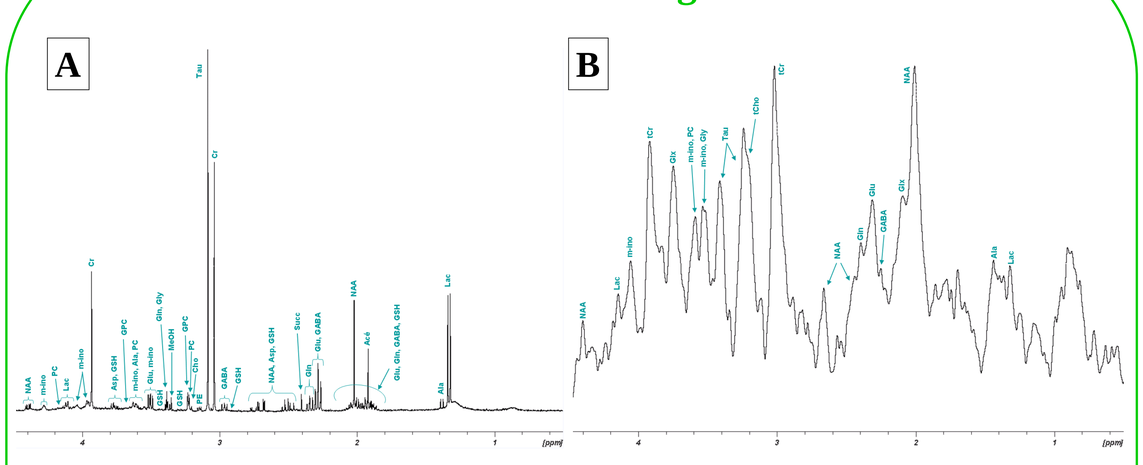


Figure 1. A) ^{1}H NMR spectrum of the aqueous extract and B) ^{1}H MRS spectrum of the hippocampus from an AppSwe Tg2576+ transgenic mouse treated with clioquinol

Lactate (Lac), alanine (Ala), glutamate (Glu), glutamine (Gln), glutamate + glutamine (Glx), γ-amino-butyric acid (GABA), acetate (Ace), N-acetylaspartate (NAA), succinate (Succ), aspartate (Asp), creatine (Cr), total creatine (tCr), taurine (Tau), phosphoethanolamine (PE), choline (Cho), total choline (tCho), phosphocholine (PC), glycerophosphocholine (GPC), glycine (Gly) and myo-inositol (m-ino). Methanol (MeOH) is a residual solvent.

Ref

[1] Hsiao K., Chapman P., Nilsen S. et al., « Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice », Science, 274:99-102 (1996)
[2] Beckonert O., Keun H.C., Ebbels T.M. et al, « Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts » Nat Protoc., 2:2692-2703 (2007)

Comparison between control and transgenic mice

	Vehicle (V)		Clioquinol (CQ)	
	NMR	MRS	NMR	MRS
Model	Non predictive	Non predictive	Predictive	Predictive
PLS-DA (axe)	1 axe	1 axe	2 axes	1 axe
Q2	0,24	0,22	0,76	0,42
Discriminating metabolites	/	/	Tau 8.5 10^{-5} Cr 8.710^{-5}	Tau 0,01 tCr 0,1

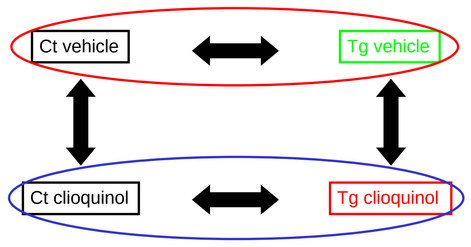


Table 1. Model evaluation and discriminating metabolites between control and transgenic mice treated with clioquinol or vehicle alone from NMR and MRS analyses.

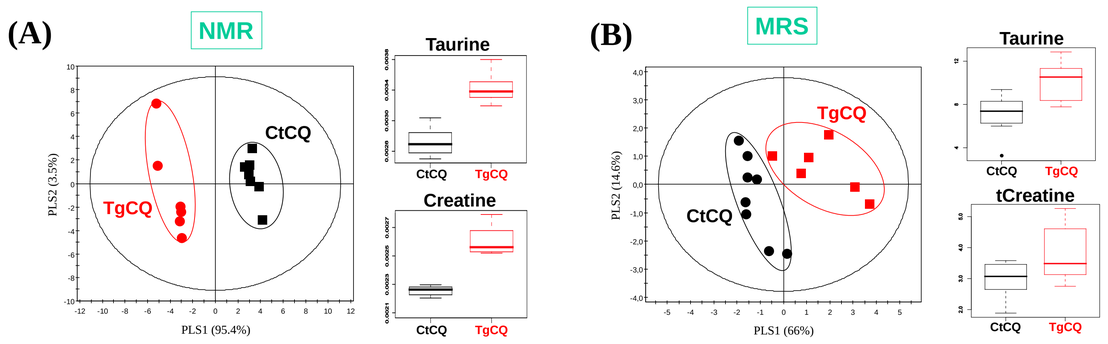


Figure 2. Score plots of PLS-DA and box plots for taurine and creatine (or total creatine) from NMR (A) and MRS (B) data for control and transgenic mice treated with clioquinol.

The results of the partial least squares-discriminant analysis (PLS-DA) presented in **Figure 2** were obtained from NMR and MRS data of control (**CtCQ**, **black**) and transgenic (**TgCQ**, **red**) mice treated with clioquinol. A good clustering is observed with both techniques. Discrimination is based on two main metabolites, taurine and creatine.

Comparison between treated mice (vehicle or clioquinol)

	Control		Transgenic	
	NMR	MRS	NMR	MRS
Model	Non predictive	Non predictive	Predictive	Predictive
PLS-DA (axe)	/	1 axe	3 axes	1 axe
Q2	/	0,15	0,92	0,44
Discriminating metabolites	/	/	Cr 0,006 Tau 0,001 Glu 0,014 NAA 0,027	PCr 0,014 tCho 0,012 Tau 0,1

Table 2. Model evaluation and discriminating metabolites between control and transgenic mice treated with clioquinol or vehicle alone from NMR and MRS analyses.

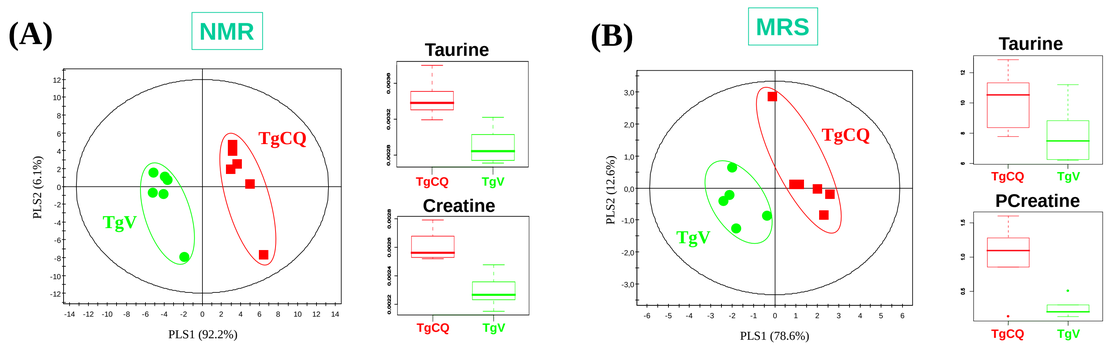


Figure 3. Score plots of PLS-DA and box plots for taurine and creatine (or total creatine) from NMR (A) and MRS (B) data for transgenic mice treated with clioquinol or vehicle alone.

The results of the PLS-DA presented in **Figure 3** were obtained from NMR and MRS data of transgenic mice treated with clioquinol (**TgCQ**, **red**) or vehicle alone (**TgV**, **green**). A good clustering is observed with both techniques. Discrimination is based on two main metabolites, taurine and creatine.

Conclusion

This study enabled the comparison of metabolites detected with *in vivo* and *in vitro* NMR in hippocampus of clioquinol-treated and untreated healthy and AD mice. Both techniques lead to similar results.

Clioquinol has no effect in control mice while it induces metabolic changes in transgenic mice. Creatine and taurine are the main discriminating metabolites. Their concentrations increase in 3 month-old transgenic mice treated with clioquinol.

Since taurine exerts a neuroprotective effect, the taurine increase is thought to prevent neuronal cell damage or cell death.