Neurochemical Changes Observed by ^{1}H MRS in Rats with Induced Age-related Early-stage Dementia

[1,2]R.Tušková, [3]B. Lipták, [3]M. Dubovický, [1]T.Liptaj, [1]S.Kašparová

[1]Slovak University of Technology in Bratislava, Faculty of Chemical and Food Technology, Institute of Analytical Chemistry, Bratislava, Slovak Republic
[2]High-Field MR Center, Department of Biomedical Imaging and Image-guided Therapy, Medical University Vienna, Vienna, Austria
[3]Slovak Academy of Sciences, Institute of Experimental Pharmacology & Toxicology, Bratislava, Slovak Republic
Email: raduss.tuskova@gmail.com

Abstract. *The aim of this study was to induce a sporadic form of age-related early-stage dementia, such as that in Alzheimer's disease (AD) by chronic injection of D-galactose and $NaNO_2$ to rats and investigate changes in brain metabolites levels using in vivo proton magnetic resonance spectroscopy. As reference methods behavioral tests were used. Localized proton magnetic resonance spectroscopy measurements in a brain showed a significant decrease in the concentration of N-acetylaspartate + N-acetylaspartylglutamate, glutamine + glutamate, and myo-inositol in the D-gal/$NaNO_2$ group compared to the control group. The dynamics of the learning process represented by the learning index in modified Morris water maze test revealed a reduction in learning in the D-gal/$NaNO_2$. The total motor activity in the Open-field test was also reduced compared to the control. We propose, that our results support the use of D-galactose in combination with $NaNO_2$ to rats to induce early-stage of dementia and could be a relevant model for the study of underlying mechanisms of sporadic forms of dementia in rats.*

Keywords: early-stage dementia, rat model, in vivo proton magnetic resonance spectroscopy, behavioral tests, D-galactose, $NaNO_2$

1. Introduction

Dementia is a broad category of brain diseases characterized by deterioration in memory, thinking, behavior and the ability to perform everyday activities. The most common type of dementia is Alzheimer's disease (AD). Two forms of AD are recognized, that are familial, which is very rare and sporadic form, where the cause is presently unknown. Several risk factors, such as lifestyle, excessive stress and excessive ingestion of sugar have been described, but the most important factor is considered to be the aging [1]. Medical treatment can be effective only for patients with early-stages, but the diagnosis is problematic. Therefore, it is very important to identify disease-specific biomarkers for early diagnosis.

D-galactose (D-gal) and $NaNO_2$ treatment is currently used in mice to induce dementia-like signs[2]. In order to understand the changes in the brain, *in vivo* proton magnetic spectroscopy (^{1}H MRS) can be used. We used single-voxel ^{1}H MRS on a 4.7 T magnet, and observed these six metabolites: N-acetylaspartate and N-acetylaspartylglutamate (*NAA+NAAG)*, creatine and phosphate creatine (*Cr+PCr*), glycerophosphocholine and phosphocholine (*GPC+PCh*), myo-inositol (*mIns*), glutamate and glutamine (*Glu+Gln*), and taurine (*Tau*). All of these metabolites are of particular interest, since they belong to specific neuronal and glial metabolic pathways, membrane constituents, and energy metabolism.

There are several experimental approaches to investigate cognitive processes in laboratory rats. Modified Morris water maze (MWM) is presently the most widely used method for the

evaluation of visual-spatial learning and memory skills [3]. Open-field (OF) test is a relevant experimental tool to analyse locomotion and habituation in a new environment [4].

The purpose of this study was to characterize in details changes in the brain metabolite levels using *in vivo* ^{1}H MRS after chronic injection of D-gal and $NaNO_2$ to adult male rats. Moreover, relevant behavioral variables with a focus on spatial learning, exploratory and anxiety-like behaviours were evaluated as well.

2. Subject and Methods

Animals

Male Wistar rats (6 months old, weight 250-320g, n = 16) were used in the study. Rats were kept under standard laboratory conditions with food and water ad libitum, and housed four in each cage on a regular light/dark cycle. Animal housing, care, and experimental procedures were conducted under the guidelines of the Animal Ethics Committee and were approved by the State Veterinary and Food Administration of the Slovak Republic. Animals were treated intraperitoneally with D-gal at a dose of 120 mg/kg and $NaNO_2$ at a dose of 20 mg/kg, dissolved in redistilled water (D-gal/$NaNO_2$ group, n = 8). The drugs were administered to animals daily for 30 days simultaneously.

In vivo ^{1}H MRS

During all MRS measurements, animals were anaesthetized by inhaling 2% isoflurane. Body temperature was maintained at 37.5°C by warm air circulating the body, and respiration was monitored during the whole experiment (SA Instruments, Inc. Stony Brook, NJ, USA). In vivo ^{1}H MRS measurements were performed on a 4.7 T horizontal bore (diameter of 12.5 cm) magnet, equipped with 400 mT/m gradients (Agilent, USA) with a transmit-volume/receive-surface combination of coils (Rapid Biomedical, Germany) intended for animals. A fast spin echo multislice sequence (FSEMS) was used for voxel localization, with the following parameters: repetition time TR= 3000 ms; echo time TE= 74.24 ms; number of acquisitions NA= 8; data matrix= 256x192; slices= 20; slice thickness= 1mm; gap= 0; field of view FOV= 40x40mm. The single-voxel localization was performed by the SPECIAL sequence, which enables the use of a short TE and preserves the full magnetization available from the selected volume of interest (VOI), with following parameters: TR = 2000 ms; TE = 4.45 ms; NA = 8x64 in combination with outer volume suppression and VAPOR water suppression. One scan without water suppression was acquired for quantification purposes. First- and second-order shim values were optimized using the FASTMAP method. Shimming resulted in an unsuppressed water spectral line width of ~10 Hz. Resulting proton spectra were analyzed using the LCModel [5]. Metabolite values that had Cramer-Rao lower bounds ≥20% were excluded from further analysis. *In vivo* ^{1}H MR spectra (Fig. 1) were obtained from an area of the hippocampus and cortex (voxel size of 4 x 4 x 4.5 mm^3).

Behavioral tests

The modified MWM was used to investigate spatial learning in experimental animals [3] and OF to analyse locomotion and habituation in a new environment.

Statistical analysis

The data are expressed as a mean ± standard deviation. Repeated measures ANOVA were used to evaluate significance between the groups. Behavioral data were statistically analyzed by STATISTICA software. Differences of $p<0.05$ were considered statistically significant in all analyses.

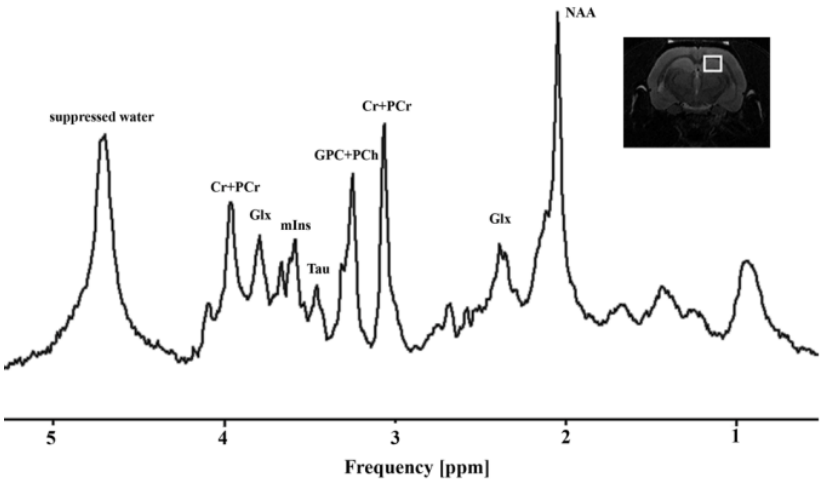

Fig. 1. A representative image and localized ^{1}H spectra from a rat brain (voxel 4x4x4.5mm^{3}) acquired on a 4.7T magnet.

3. Results

In vivo ^{1}H MRS

A quantification of absolute concentrations of metabolites from ^{1}H MRS in the D-gal/$NaNO_2$ versus the control group showed a significant decrease in the concentration of NAA+NAAG ($p<0.002$), as well as a significant decrease in the concentration of mIns ($p<0.008$) and Glu+Gln ($p<0.02$) (Fig. 2).

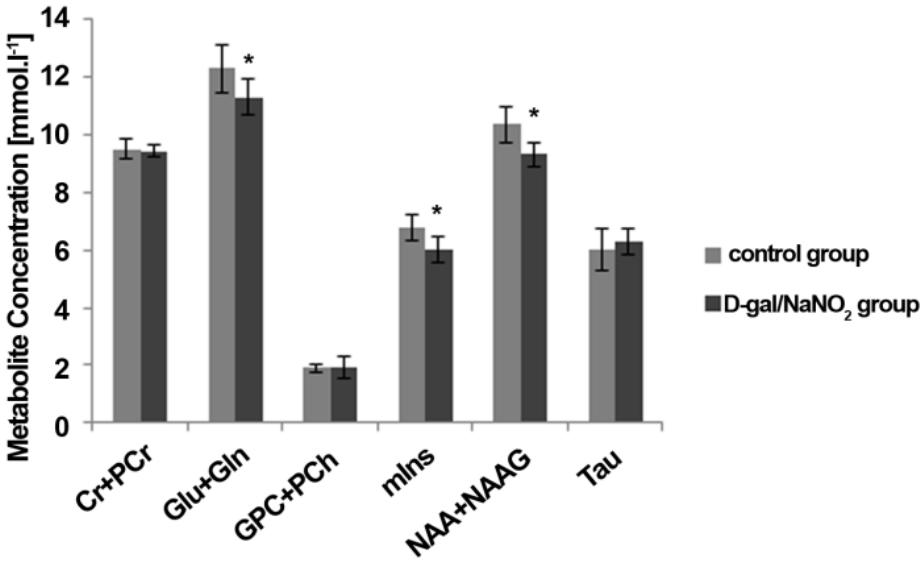

Fig. 2. A bar graph showing absolute concentrations of metabolites in the D-gal/$NaNO_2$ versus the control group.

Behavioral tests

The mean learning index (MWM) calculated for the D-gal/$NaNO_2$ group was lower compared to controls ($p<0.059$) and the rapidity of learning was significantly lower in the D-gal/$NaNO_2$ group compared to control group ($p<0.044$). The motor activity (OF) of animals did not continuously decrease (they did not habituate), as expected in the repeated open-field test exposures.

4. Discussion

It has recently been reported that the process in mice treated with D-gal and $NaNO_2$ resembles observations in humans with some age-related dementia. The doses of D-gal in combination with $NaNO_2$ for intraperitoneal injection in mice are well known. We had to test dose for rats. The dosage of D-gal in rats was determined from past experiments. We tested more doses of $NaNO_2$ and the most suitable dosage for long term and survival intraperitoneal administration was determined at 20mg/kg. Numerous *in vivo* and *in vitro* ^{1}H MRS studies of AD and transgenic animal models have been performed and have shown the typical neurochemical profile demonstrated by a decrease in brain NAA and Glu and an increase in mIns. We observed similar changes in our rat brain model for the metabolites NAA+NAAG and Glu+Gln. Decreased brain NAA concentration may reflect neuronal dysfunction and decreased Glu level can mirror disturbed learning and memory systems. A decrease of mIns concentration was observed, which is contrary to some *in vivo* and *in vitro* ^{1}H MRS studies that have found increased mIns. As was suggested by Mlynarik et al. (2012), an increase in mIns can be detected only when the pathology is severe. The results from the behavioral study reflect in some extent the results obtained by means of MRS approaches.

5. Conclusions

In good agreement with previous *in vivo* and *in vitro* ^{1}H MRS findings, our *in vivo* ^{1}H MRS at 4.7T observed decreased NAA and Glu in the hippocampus of rats, so we concluded that we induced age-related dementia (prodromal stage of dementia, such as AD) by mentioned substances. The results of the present work may at least partially provide some evidence for a possible role for MRS in early diagnosis and for surrogate biochemical markers to monitor disease progression and therapeutic response.

Acknowledgements

This work was supported by the grant agency VEGA, grant no.: 2/0084/11 and 2/0168/15, and by the Support for Young Researchers, Slovak University of Technology in Bratislava.

References

[1] Querfurth HW, Laferla FM. Alzheimer's Disease. *J. Med.*, pp. 329–344, 2011.

[2] Zhang D, Liu G, Shi J, Zhang J. Coeloglossum viride var. bracteatum extract attenuates D-galactose and NaNO2 induced memory impairment in mice. *J. Ethnopharmacol.*, vol. 104, no. 1–2, pp. 250–6, Mar. 2006.

[3] Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat. Protoc.*, vol. 1, no. 2, pp. 848–58, Jan. 2006.

[4] Prut L., Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *Eur. J. Pharmacol.*, vol. 463, pp. 3–33, 2003.

[5] Provencher SW. Automatic quantitation of localized in vivo 1H spectra with LCModel. *NMR Biomed.*, vol. 14, no. 4, pp. 260–4, Jun. 2001.