Early post-ischemic neuroprotective mechanisms: a MR spectroscopic imaging study on PPARβ-deficient mice

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INTRODUCTION

PPARβ (peroxisome proliferator-activated receptor β) is a ligand-activated nuclear receptor belonging to the PPAR family known as metabolic sensors. Recent studies have shown the neuroprotective role of PPARβ in several brain diseases [1]. Moreover, it has been shown that PPARβ-deficient mice exhibit a more extensive lesion than wild-type (WT) mice after a permanent middle cerebral artery occlusion that induces focal ischemia, as soon as 3 hours post-ischemia [2]. PPARβ thus appears to be involved in the events that occur early after ischemia. However, no *in vivo* metabolic study has been performed on ischemic PPARβ-deficient mice. Therefore the aim of this study is to detect the metabolites involved in the neuroprotection through an early neurochemical comparison between PPARβ-deficient and WT mice after a transient ischemia of the middle cerebral artery using MR spectroscopic imaging (SI). The use of SI on an ultra-high field 14.1 T magnet in this study provides a sensitive means to map the *in vivo* metabolite concentrations.

METHODS

Four PPARβ-knockout mice and four wild-type mice of mixed background (~25 g) underwent 30 minutes endoluminal middle cerebral artery occlusion (MCAo) by the filament technique [3]. Laser-Doppler flowmetry with a flexible probe fixed on the skull was used to assess the reduction in regional blood flow and its reestablishment up to 10 minutes after terminating ischemia. SI studies were performed three hours after the end of ischemia on a horizontal 14.1 T magnet (Varian/Magnex) using a custom-built quadrature coil. After shimming with FASTMAP, SI was performed in the striatum region using a modified SPECIAL sequence (TR = 2.5 s, TE = 2.8 ms) with phase encoding in the horizontal plane [4]. The nominal size of the volume of interest (VOI) was 5×7.5×1.5 mm^3. Two averages of 32×32 phase encoding steps were acquired in a FOV of 19×19 mm^2, resulting in a voxel size of 0.6×0.6×1.5 mm^3 (0.5 µl). The *k*-space data were then filtered with a Gaussian function in two spatial directions. The neurochemical profile was determined from the spectra of individual voxels using LCModel. Reference water signals were measured using the same protocol without water suppression and with TR=1.5 s.

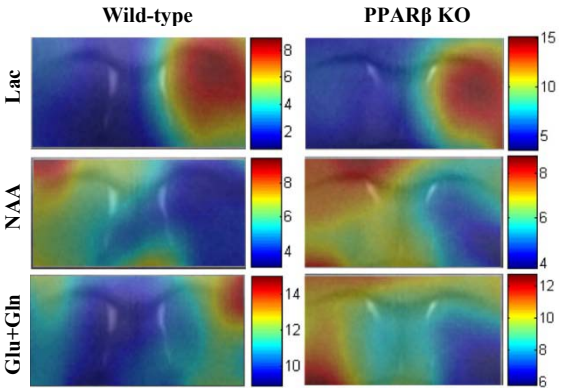

Figure 1: *Metabolite maps (color) superimposed on anatomical images (grayscale) obtained 3 hours after ischemia. Metabolite maps were obtained by spatial interpolation with a Gaussian convolution of the metabolite concentration. Each map has its own scale based on the highest and lowest value on the map.*

RESULTS AND DISCUSSION

At 3 hours after ischemia, no lesion could be detected on T_2–weighted images. After shimming the VOI, the water linewidth was ~ 28 Hz. Maps of lactate (Lac), NAA and the total of glutamine and glutamate (Glu+Gln) superimposed on T_2–weighted images of the mice brain are displayed in Fig1. A visible increase of lactate correlated with an NAA decrease is apparent in the lesion. These visible metabolite alterations were used for localizing the lesion site. Interestingly, it is apparent that the Glu+Gln concentration in the lesion site is affected differently in PPARβ-deficient compared to WT mice: In WT mice, the Glu+Gln concentration slightly increases in the lesion site. On the contrary, the Glu+Gln concentration decreases in the lesion of PPARβ-deficient mice.

For comparison, four quantified voxels were selected inside the lesion as well as in the contralateral side of the brain in each mouse, resulting in selected volumes of 2 µl. Fig. 2 shows the metabolite concentrations obtained for both mouse groups. A significantly lower level of Glu+Gln was observed in the lesion of the PPARβ-deficient compared to WT mice correlated with lower levels of glutamine (Gln) and glutamate (Glu). Furthermore, a significantly two-fold higher level of Lac was detected in the PPARβ-deficient mice. No significant difference in the contralateral region of the brain was detected between the two mice groups.

These results support the hypothesis that PPARβ-deficient mice might be more sensitive to ischemia because of an impaired energetic metabolism that could affect the Lac level and energetic processes such as Glu uptake by astrocytes, which leads to a higher degradation of the glutamate and a lower level of Gln formation.

The use of SI in this study allowed a sensitive localization of the lesion site through early post-ischemic metabolite changes when no lesion could be detected on MR images, insuring an optimal evaluation of *in vivo* metabolite concentration alterations that were caused by the neuroprotection deficit of PPARβ-deficient mice.

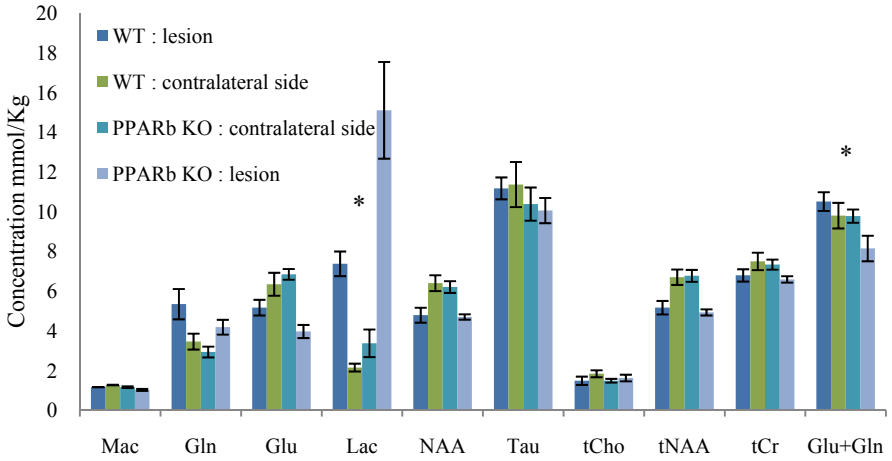

Figure 2: *Metabolite concentration (mean ± SEM) for a 2 µl volume in both lesioned and contralateral brain sides in the wild-type and PPARβ-knockout mice. All metabolites included in this comparison had Cramer-Rao lower bonds lower than 15%. Significance is represented by * ($p < 0.05$).*

References: 1. Collino and al., Ther Adv Cardiovasc Dis. 2008 Jun; 2(3):179-97. 2. Pialat and al., NMR Biomed. 2007 May; 20(3):335-42. 3. Huang Z et al., Science. 1994 Sep23; 265(5180):1883. 4. Mlynarik V. and al., Magn Reson Med. 59:52, 2008.

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