Hippocampal neurochemical changes in neonatal mouse model of phlebotomy-induced anemia

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INTRODUCTION

Neonatal anemia is a complication of phlebotomy-induced blood loss in preterm newborn infants [1]. Uncompensated blood loss results in iron deficiency, which affects brain development, especially the vulnerable hippocampus. Such iron deficiency during brain development may cause long-term hippocampally based cognitive deficits [2]. The aim of this study was to investigate neurochemical changes in hippocampus using a phlebotomy-induced anemia model in neonatal mice.

METHODS

Mice underwent blood draw (3.5 µL/g body weight) two or three times a day beginning at postnatal day 3 (P3) until their hematocrit decreased below 25% and were then bled only once a day to maintain the anemic state until day P14. Spontaneously breathing mice were anesthetized with the gas mixture N_2O : O_2 = 1 : 1 and 1.0 – 1.2% isoflurane. In vivo ^{1}H NMR spectra were acquired from 3 µL VOI centered in hippocampus of both anemic and unbled control mice on day P14. Measurements were performed at 9.4T using FASTMAP shimming and ultra-short TE STEAM (TE = 2 ms) localization sequence combined with VAPOR water suppression [3,4]. Metabolites were quantified using LCModel with the spectrum of fast relaxing macromolecules included in the basis set.

RESULTS

Periodic blood draws resulted in anemia with significantly decreased ($p < 0.0001$) hematocrit of 20 ± 2% relative to unbled control mice (Htc = 33 ± 3). Body weight was slightly lower in the anemic group (5.5 ± 1.1 g) relative to control (6.4 ± 1.3 g). Consistently achieved spectral quality (Fig. 1) enabled reliable quantification of 15 brain metabolites (Fig. 2). Small, but significant changes in *myo*-inositol (*myo*-Ins; 0.4 µmol/g, +22%), lactate (Lac; 0.6 µmol/g, +37%) and phosphoethanolamine (PE; -0.5 µmol/g, -9%) were observed in anemic mice relative to controls (Fig. 2).

DISCUSSION

Observed changes in *myo*-Ins and PE indicate altered myelination within the hippocampus of anemic mice, which is in agreement with decreased myelination observed within the hippocampus of iron deficient rats [5]. Increased lactate levels may indicate a shift in the redox potential resulting from iron deficiency. These results suggest that uncompensated phlebotomy-induced blood loss is a risk factor, especially for preterm infants, and has implications on blood transfusion practices.

REFERENCES: **1.** Rao et al., *Clin Perinatol* 2009: 36, 27; **2.** Rao et al., *Semin Fetal Neonetal Med* 2007: 12, 54; **3.** Tkac et al., *Magn Reson Med* 1999: 41, 649; **4.** Tkac et al., *Magn Reson Med* 2004: 55, 979; **5.** Rao et al., *Pediatr Res* 2012: DOI: 10.1038/pr.2012.143.

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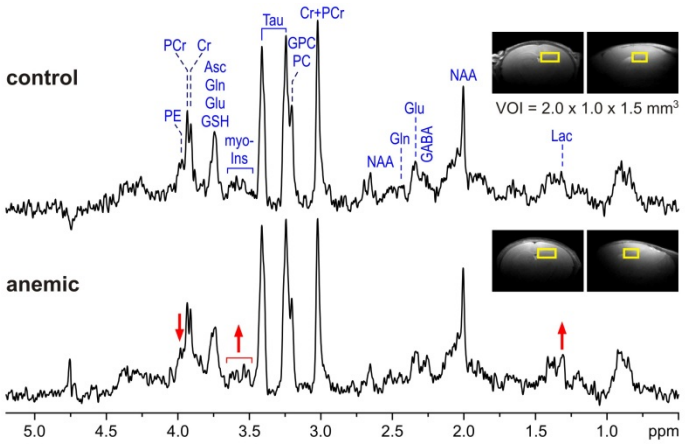

Fig. 1 In vivo 1H NMR spectra acquired from the hippocampus of anemic and control mice. STEAM, TE = 2 ms, TR = 5 s, NT = 240, VOI = 3 µL. No water removal or baseline corrections were applied.

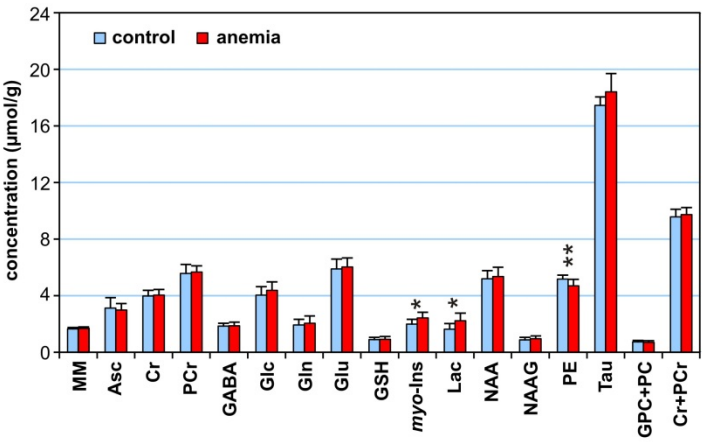

Fig. 2 Comparison of hippocampal neurochemical profiles of anemic (N = 13) and control mice (N = 9). LCModel analysis, simulated basis set, water signal reference assuming 85% brain water content.