Long-term dosage effects in iron repletion treatment on neurochemical profiles and gene expression in rat model of neonatal iron deficiency

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INTRODUCTION

Neonatal iron deficiency (ID) is common in pregnancies complicated by maternal anemia, diabetes mellitus and hypertension [1]. Long-term cognitive deficits, suggesting hippocampal and prefrontal cortex (PFC) dysfunction are the sequelae of neonatal iron deficiency in humans and rats [2]. These deficits persist despite correction of brain iron deficiency and are associated with neurochemical alterations at adulthood [3]. The aim of this study was to investigate the long-term effects of iron repletion therapy on neurochemical profiles and gene expression in ID rat model using two different iron dosage protocols.

METHODS

Iron deficiency in rats was induced using ID diet (3 mg/kg) during pregnancy through postnatal day (P) 7. Iron was supplemented from P8 using 40 mg/kg (ID-40, standard dose) or 400 mg/kg (ID-400, high dose) iron diet from P8 to P21, followed by 40 mg/kg diet until adulthood. In vivo ¹H NMR spectra were acquired from the hippocampus and PFC of ID-40, ID-400 and iron sufficient (IS, 40 mg/kg diet throughout life) rats (N = 7 - 8) on P90 using ultra-short TE STEAM sequence (TE = 2 ms, TR = 5 s) combined with VAPOR water suppression at 9.4T [4]. The mRNA expressions of myelin basic protein-1 (Mbp1), profilin-1 (Pfn1) and calcium/calmodulin-dependent protein kinase II alpha (CamKIIa) genes were determined in the hippocampus and PFC using qPCR method (N = 8).

RESULTS

Seventeen brain metabolites were consistently quantified from ¹H NMR spectra acquired from the hippocampus and PFC on P90 (Fig. 1A and B). Differences in metabolite concentrations between iron treated rats (ID-40 or ID-400) and IS controls were not observed for any metabolite in PFC (Fig. 1C); however, small but significant (p < 0.05) differences in NAA, NAAG, PE and GPC+PC concentrations were detected in the hippocampus (Fig. 1D). Standard dose treatment resulted in decreased NAA (-6%) and PE (-15%) and increased NAAG (+37%) and GPC+PC (17%) and relative to IS controls. Decreased PE (-15%) and increased GPC+PC (27%) were also found in high dose treatment ID-400 group relative to IS group. Similar to the NMR spectroscopy results, Mbp1, Pfn1 and CamKIIa mRNA expressions in both ID groups were comparable to the IS group in the PFC. But in the hippocampus, Mbp1 and Pfn1 mRNA expressions were increased in both iron treatment groups relative to IS controls (IS < ID-40 < ID-400, p < 0.05). CamKIIa was increased in ID-400 relative to the IS group (p<0.05).

DISCUSSION

Changes in the neurochemical profiles observed in neonatal ID rats [5] were almost normalized by the iron repletion therapy. Long-term effects of the neonatal ID and subsequent iron supplementation were still observable at P90 in hippocampus, but not in PFC. In vivo ¹H NMR spectroscopy data were in very good agreement with the gene expression results, which suggest altered phospholipid metabolism (GPC+PC) and neurite formation (Pfn1 and CamKIIa) as well as the decreased myelination (PE) and compensatory upregulation of myelin protein gene Mbp1 [6]. In addition, increased NAAG levels in ID-40 group may indicate altered glutamatergic neurotransmission in the formerly ID hippocampus [7]. Region-specific neurochemical alterations may be responsible for the persistent hippocampus-based cognitive deficits due to neonatal iron deficiency. Observed dose-dependent effects of iron repletion protocol have to be taken into account for optimizing the therapy of neonatal ID.


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Fig. 1 In vivo ¹H NMR spectra (A, B) and corresponding neurochemical profiles (C, D) of prefrontal cortex and hippocampus.