^{1}H-[^{13}C]-NMR Investigations of Glutamatergic and GABAergic Metabolism in Aluminium Chloride Model of Alzheimer's Disease

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INTRODUCTION: Alzheimer's disease (AD) is the most common form of neurodegenerative disorders. AD is characterized by the progressive loss in memory and accumulation of amyloid beta peptide. Synaptic dysfunction and neurotransmitter deficit have been reported in AD subjects[1]. Chronic aluminium treatment leads to similar pathological symptoms as that in AD[2]. Glutamatergic and GABAergic neurons together constitute more than 90% of total cortical synapses[3] and contribute to the majority of energy metabolism in the cerebral cortex[3]. The present study investigates the effects of chronic aluminum exposure on glutamatergic and GABAergic metabolism in mouse cortex by using ^{1}H-[^{13}C]-NMR spectroscopy in conjunction with infusion of labeled glucose.

MATERIALS AND METHODS: All animal experiments were performed under approved protocols by the Institutional Animal Ethics Committee. C57Bl-6 mice were treated with Aluminium chloride once in a day (4 mg/kg, i.p) for 30 days. For metabolic measurements, overnight fasted mice were anesthetized using urethane (1.5 g/kg, i.p.). [1,6-$^{13}C_2$]Glucose was administered using a bolus variable infusion schedule[4] after 45 minutes of induction of anesthesia. Blood was collected and head was frozen *in situ* using liquid nitrogen at the end of infusion. Metabolites were extracted from frozen cortical tissue[5]. Concentration and percentage ^{13}C enrichment of cortical amino acids were measured in ^{1}H-[^{13}C]-NMR spectra of tissue extracts acquired at 600 MHz spectrometer[6]. ^{13}C Turnover of cortical amino acids from [1,6-$^{13}C_2$]glucose was analyzed using a three compartment metabolic model to determine the glutamatergic and GABAergic fluxes upon $AlCl_3$ exposure[2].

RESULTS AND DISCUSSIONS: Quantification of cortical metabolites using ^{1}H-[^{13}C]-NMR spectrum (Fig. 1, ^{1}H-[^{12}C+^{13}C]) did not reveal any significant alteration in neurochemical profile following chronic aluminium treatment. The time course of labeling of cortical metabolites from aluminium treated mice shows labeling of glutamate, glutamine, GABA, aspartate, alanine at different carbons which reaches to a steady state value during 60 to 90 min of glucose infusion (Fig. 1, ^{1}H-[4x^{13}C]). ^{13}C Labeling of cortical amino acids from [1,6-$^{13}C_2$]glucose was used for metabolic modeling to derive the metabolic rates (Fig. 2A). Chronic exposure of $AlCl_3$ was found to reduce the glutamatergic TCA cycle (Control 0.72±0.09; $AlCl_3$ 0.54±0.06 µmol/g/min) and glutamate-glutamine cycle (Control 0.28±0.03; $AlCl_3$ 0.21±0.02 µmol/g/min) fluxes. Similarly, the GABAergic fluxes (TCA cycle and neurotransmitter cycle) were also reduced but to a lesser extent than glutamatergic. Although there was no perturbation in neurochemical homeostasis upon long term aluminium exposure but neuronal activity was severely impaired. Previous studies in human AD patients[7] and in mouse model of AD[8] has shown glucose hypometabolism. The finding of reduced glucose consumption with chronic aluminium treatment (0.29±0.02 vs 0.45 ±0.2 µmol/g/min) is in good accordance with previous findings in AD. Hence, chronic aluminium exposure decreases the neuronal function similar to AD patients and in animal models of AD. Thus it may be used for understanding the mechanism of AD and evaluation of the efficacy of potential AD drugs.

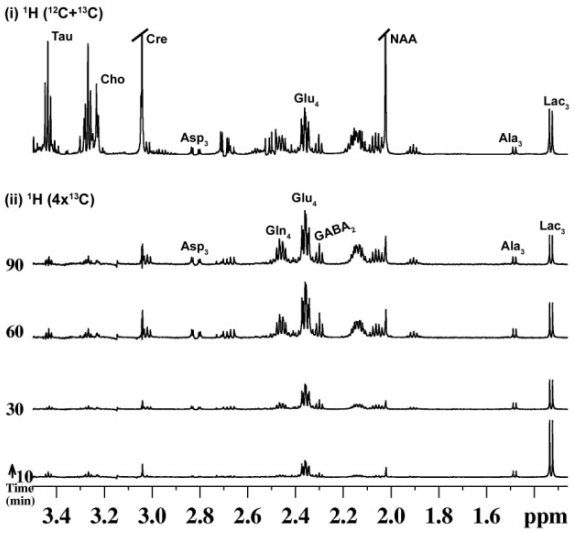

Fig. 1 ^{1}H-[^{13}C]-NMR spectra from cerebral cortex of mice treated with $AlCl_3$

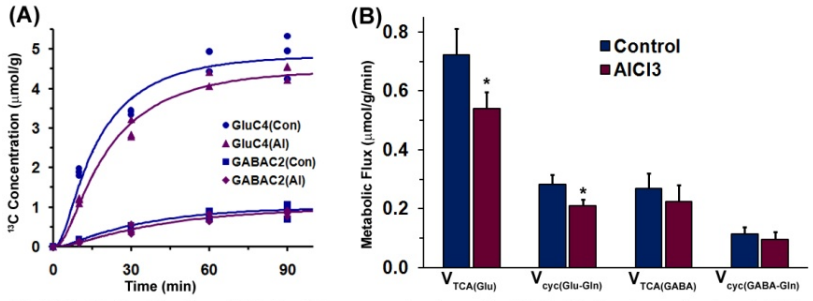

Fig. 2 (A) Fit of metabolic model to the ^{13}C turnover of amino acids, (B) Metabolic rates under chronic $AlCl_3$

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