^{1}H-[^{13}C]-NMR Study of Cerebral Energy Metabolism under sub-Acute Exposure of Aluminium Chloride in Mice: Implications for Dementia

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Introduction: Aluminium (Al), an important environmental toxin, disturbs iron homeostasis, leading to increase in the intracellular pool of labile iron and a concomitant rise in reactive oxygen species[1]. As the ionic size of Al resembles Ca^{2+}, it renders Ca^{2+} signaling networks dysfunctional due to its ability to bind with Ca^{2+}-dependent proteins. The Various ion channels have been shown to be inactivated by micromolar quantities of Al. Aluminium has been implicated in Alzheimer's disease, amyotrophic lateral sclerosis Parkinson's disease, anemia and encephalopathy. Although molecular aspects of Al toxicity are becoming evident, its interaction with the various metabolic pathways that mediate the synthesis of ATP and the generation of a reducing environment in the cell has yet to be unravelled[2]. The present study investigates the cerebral metabolism in mice under sub-acute exposure of $AlCl_3$ by using ^{1}H-[^{13}C]-NMR spectroscopy together with infusion of [1,6-$^{13}C_2$]glucose.

Materials and Methods: All animal experiments were carried out with approved protocols from the Institutional Animal Ethics Committee. Two groups of male C57BL6J mice (2 month) were used for the study. Group (i) normal saline (n=5), Group (ii) AlCl3 treatment group (40 mg/kg) (n=6). Mice were treated with AlCl3 (40 mg/kg, i.p.) for 7 days. For metabolic measurements, overnight fasted mice were anesthetized with urethane (1.5 g/kg), i.p.). [1,6-$^{13}C_2$]Glucose was administered via tail vein into mice for 10 min using a bolus-variable rate infusion protocol[3]. Blood was collected and head was frozen *in situ* into liquid nitrogen at the end of the infusion. Metabolites were extracted from frozen brain tissue (cerebral cortex, hippocampus and striatum)[4]. Concentration and percentage ^{13}C enrichment of cerebral amino acids were measured in ^{1}H-[^{13}C]-NMR spectrum (Fig. 1A) of tissue extracts acquired at 600 MHz spectrometer[5]. Glutamatergic and GABAergic neuronal glucose oxidation rate was calculated from the initial rate of labeling from [1,6-$^{13}C_2$]glucose as described earlier[6].

Results and Discussion: The level of GABA, NAA and choline was found to be increased significantly ($p<0.05$) in the cerebral cortex while the level of glutamate was increased in striatum of aluminium treated mice as compared with normal saline treated control, suggesting that sub-acute exposure of aluminium perturbs the neurometabolites homeostasis. ^{13}C labeling of Glu_{C4}, $GABA_{C2}$, Gln_{C4} and Glu_{C3} was found to increase in the cerebral cortex, hippocampus and striatum indicating that both, glutamatergic and GABAergic metabolism were enhanced with sub-acute exposure of $AlCl_3$ (Fig. 1B). The glucose oxidation associated with glutamatergic and GABAergic neurons was found to increase with sub-acute Al exposure. The increase in glutamatergic metabolism follows the order: hippocampus < cerebral cortex < striatum while GABAergic neuronal metabolism increases in the order hippocampus < striatum < cerebral cortex. These data indicate that sub-acute exposure of aluminium enhances excitatory and inhibitory neuronal activity across brain regions in mice. The increased neuronal metabolism/firing due to aluminium chloride exposure would result in excitotoxicity and neural death that may lead to dementia in long term.

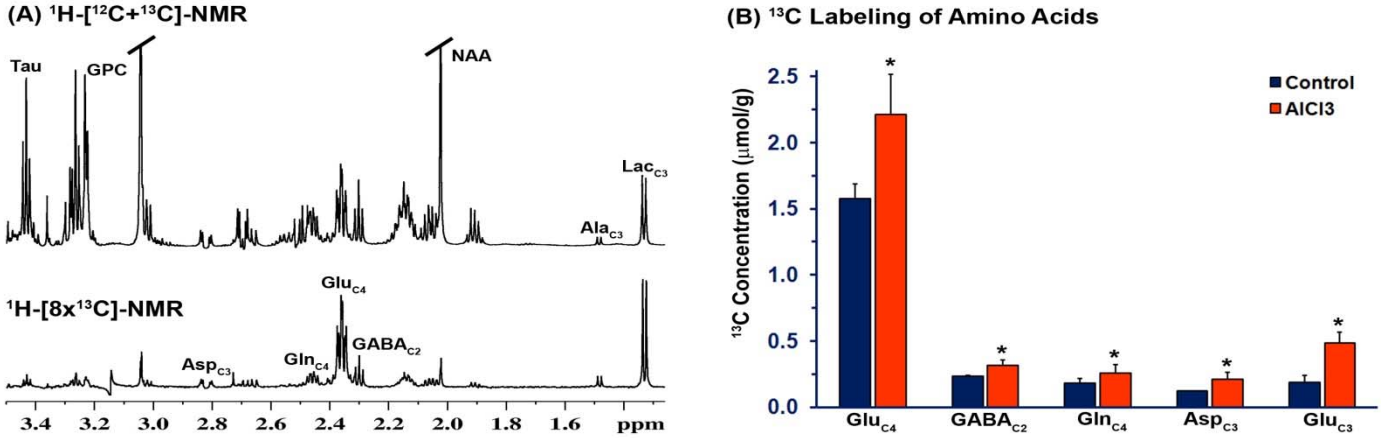

Fig. 1 (A) ^{1}H-[^{13}C]-NMR spectra, **(B)** Concentration of ^{13}C Labeled amino acids in striatal extract of mouse treated with $AlCl_3$

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