^{1}H-[^{13}C]-NMR Study of Brain Energy Metabolism in $AlCl_3$ Model of Alzheimer's Disease: Improvement of Energy Metabolism with Rasa-Sindoor Intervention

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TARGET AUDIENCE: Researchers and clinicians interested in brain energy metabolism and intervention in Alzheimer's disease.

INTRODUCTION: Alzheimer's disease (AD) is one of the most common neurodegenerative disorders, characterized by neuritic plaques and neurofibrillary tangles, and marked with loss of cognitive functions and memory impairment. At present, there is no effective strategy for management/treatment of AD. Genetic and chemical models of rodents have been used to study the pathology of AD[1,2]. Chronic exposure of $AlCl_3$ in mice resulted in decreased excitatory and inhibitory metabolic activities in different brain regions[3]. *Rasa-Sindoor (RS),* an organometallic derivative of mercury, has been used as an Ayurvedic medication in Indian traditional therapy. RS has been shown to prevent accumulation of heat shock proteins, suppress apoptosis and improved the ubiquitin-proteasomal system in drosophila model of different neurodegenerative disorders[4]. In the present study, we evaluated the effects of RS on glutamatergic and GABAergic pathways in the $AlCl_3$ model of AD using ^{1}H-[^{13}C]-NMR spectroscopy together with an infusion of [1,6-$^{13}C_2$]glucose.

MATERIALS AND METHOD: All animal experiments were performed under approved protocols by the Institutional Animal Ethics Committee. Four groups of C57BL6 (2 months old) mice: Group (i) Control+carboxymethyl cellulose (CMC, n=5), Group (ii) Control+RS (n=6), Group (iii) $AlCl_3$+CMC (n=6) and Group (iv) $AlCl_3$+RS (n=6), were used for the study. Mice in Group (iii) and (iv) received $AlCl_3$ (40 mg/Kg, i.g.) for 60 days that was followed by CMC and RS (2 g/kg, i.g.) treatment for 30 days, respectively. Mice in Group (i) and (ii) received normal saline followed by CMC or RS intervention for same period. Learning and memory of mice were assessed using Morris Water Maze (MWM) test[5]. For metabolic measurements, urethane (1.5 g/Kg, i.p.) anesthetized mice were infused with [1,6-$^{13}C_2$]glucose for 10 min[6]. Mice head was frozen in-situ in *liq.* N_2, and metabolites were extracted from frozen hippocampal and cortical tissue[7]. The concentration and ^{13}C labeling of amino acids were measured in ^{1}H-[^{13}C]-NMR spectrum of tissue extracts[8]. Cerebral metabolic rate of glucose oxidation ($CMR_{Glc(Ox)}$) was calculated from the amino acids labeling from [1,6-$^{13}C_2$]glucose[9].

RESULTS AND DISCUSSION: The escape latency in MWM test was significantly (p=0.036) higher in $AlCl_3$ treated mice (75.7±10.1 s) as compared to controls (47.0±8.8 s) suggesting that exposure of $AlCl_3$ impairs memory in mice (Fig. 1). RS intervention was found to decrease the escape latency (41.2±6.8 s) to the control level indicating improvement in memory with RS in $AlCl_3$ treated mice. Treatments of $AlCl_3$ and RS did not affect the homeostasis of metabolites in cerebral cortex and hippocampus. The ^{13}C labeling of Glu_{C4} (11.1±1.0 vs 14.6±0.6%, p=0.0004), $GABA_{C2}$ (4.6±1.0 vs 7.6±1.0%, p=0.004) and Gln_{C4} (3.0±0.4 vs 4.9±0.6%, p=0.0009) from [1,6-$^{13}C_2$]glucose was found to be decreased significantly in hippocampus with chronic $AlCl_3$ treatment (Fig. 2). RS intervention restored Glu_{C4} (14.3±1.1%), $GABA_{C2}$ (6.7±0.8%) and Gln_{C4} (4.6±0.6%) labeling to the control level. $CMR_{Glc(Ox)}$ associated with glutamatergic and GABAergic neurons was found to be reduced significantly (p<0.01) in $AlCl_3$ treated mice (Fig. 3). These values were restored to the control level in mice treated with RS. Similar results were observed for cortical region. Neuronal glucose metabolism is stoichiometrically coupled with neurotransmitter cycling in brain[10]. Hence, reduced $CMR_{Glc(Ox)}$ indicates decreased synaptic activity following chronic $AlCl_3$ treatment. The finding of decreased labeling of Gln_{C4} is consistent with reduced synaptic transmission in $AlCl_3$ treated mice. Moreover, finding of increased $CMR_{Glc(Ox)}$ in $AlCl_3$ treated mice suggests improved neuronal activity with RS intervention. These data suggest that RS intervention has potential for management of memory and cognitive functions in AD.

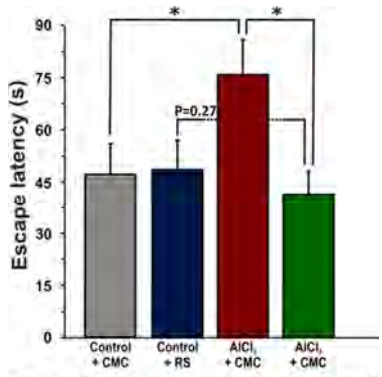

Fig. 1 Memory of mice upon various treatments

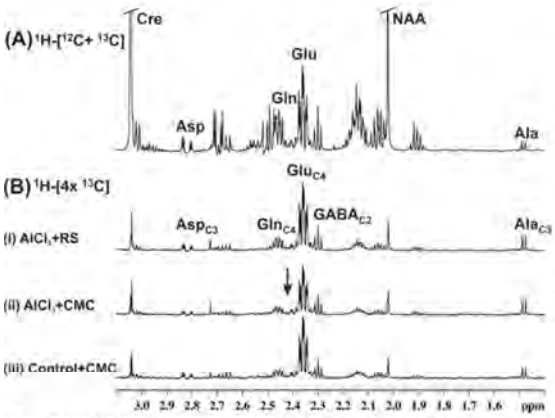

Fig. 2 ^{1}H-[^{13}C]-NMR spectra of hippocampal extract upon different treatments

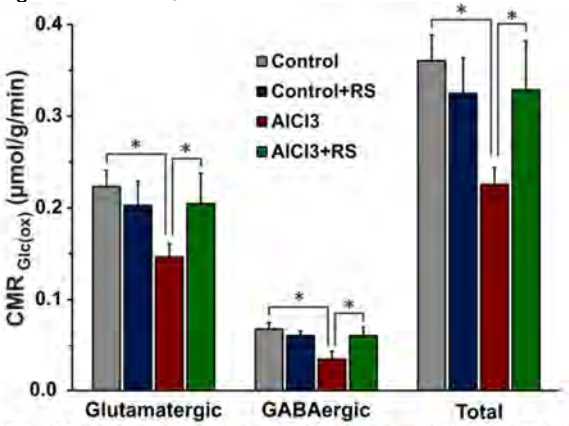

Fig. 3 Cerebral metabolic rate of glucose oxidation (CMR_{Glc}) upon different interventions

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