Glutamatergic and GABAergic neurotransmission in Manganism using $^{13}$C NMR Spectroscopy

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Introduction
Manganese is an essential element and plays important role in many biological functions. Many workers of Manganese mines or factories suffer from a disease which is symptomatically similar to idiopathic Parkinson's disease commonly known as Manganism. The mechanism of progression of Manganism is only speculated, but not much has been explored in the area of brain energy metabolism. The objective of this study was to evaluate the effect of manganese on glutamatergic, GABAergic and astroglial functions by using a novel approach of co-infusion of [U-$^{13}$C$_6$]glucose and [2-$^{13}$C]acetate in conjunction with $^{13}$C NMR spectroscopy.

Materials and Methods
All animal experiments were performed under protocols approved by Institute Animal Ethics Committee. Two group of C57BL/6 mice (Group A: Manganese (n=5); Group B: Control (n=5)) were used. Group A mice were treated with manganese chloride (40 mg/kg, i.p) for 21 days while the control mice received normal saline. In vivo $^1$H NMR spectra were acquired on 600 MHz (Bruker AVANCE) NMR microimager/spectrometer from striatum and thalamus/hypothalamus using STEAM localisation technique. For metabolic study, overnight fasted mice were infused with [U-$^{13}$C$_6$]glucose and [2-$^{13}$C]acetate$^1$. At the end of the experiment, brain was frozen in situ in liquid nitrogen and metabolites were extracted from frozen brain regions$^2$. The $^1$H-$[^{13}$C$]$-NMR and $^{13}$C-$[^1$H$]$-NMR NMR spectra were acquired from tissue extracts for the measurements of $^{13}$C enrichment and isotopomer of amino acids$^3$. The $^{13}$C labelling of glutamate, GABA and glutamine from [U-$^{13}$C$_6$]glucose and [2-$^{13}$C]acetate were calculated from the measured percent enrichment and isotopomers.

Results and Discussions
In vivo $^1$H NMR spectrum suggested a decrease in the concentration of NAA, taurine and choline in striatum suggesting neurodegeneration in manganese treated mice. Quantification of metabolites using ex vivo NMR spectroscopy in tissue extracts indicated a decrease in glutamate and glutamine level further suggesting loss in neuronal as well as astroglial cells in striatum, thalamus / hypothalamus and olfactory bulb after chronic manganese treatment. Moreover, cerebral metabolic study revealed that the $^{13}$C labelling of Glu$_{C4}$ and Gln$_{C4}$ from [U-$^{13}$C$_6$]glucose and [2-$^{13}$C]acetate was decreased significantly in thalamus (P<0.004) and striatum (P<0.003) indicating an impairment in glutamatergic and GABAergic TCA cycle and glutamatergic neurotransmission after treatment of manganese. Further, labelling of GABA$_{C2}$ from glucose and acetate was also reduced significantly in thalamus indicating attenuation in inhibitory function. The reduction in glutamatergic, GABAergic and astroglial function in thalamus and striatum may be related with the manganism.


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