Neurochemical changes in scopolamine induced memory impairment in the mouse are detectable by in vivo magnetic resonance spectroscopy

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INTRODUCTION

Scopolamine (SCP) an amnesic agent, which is an acetylcholine (ACh) receptor antagonist, has been employed to make a memory impairment model in rodents.. However, molecular details of SCP action and its effects on signaling pathways remain undefined. [1, 2] It is known that *in vivo* NMRS is noninvasive and an excellent tool to detect the levels of choline containing compound in the brain. In addition MRS has been used to characterize diseases such as Alzheimer's Dementia that exhibit memory loss. The present study is aimed at assessing neurochemical changes in SCP induced memory impairment as a function of both dose and time after administration using *in viv*o proton MR spectroscopy.

MATERIALS AND METHODS

Adult male Swiss albino mice (12~16 weeks of age; n=32) were used. Scopolamine hydrobromide was dissolved in 0.9% normal saline and administered (i.p). Mice were divided into four groups: SCP 0, 1, 3, and 5 (mg/kg). All of the mice underwent ^{1}H MR spectroscopy twice: at 30 min and 72 hours after the injection of a solution of SCP. The MRI/MRS studies were carried out on a horizontal 9.4 T/31 cm magnet (Agilent, Palo Alto, CA). The VOI whose size is 7.5 µl (1.5 × 2.0 × 2.5 mm^3) was adjusted for coverage of the left hippocampal region. PRESS sequence was used to localize the VOIs: TR/TE = 3000/19 ms, average = 512, complex data points = 4096, spectral width = 4000 Hz. All of the procedures for MR scanning were completed within 1 hour. All of the *in vivo* ^{1}H MR spectra were processed with LCModel in order to calculate metabolite concentrations from a fit to the experimental spectrum, based on simulated basis set. The data for each metabolite were tested for homogeneity of variance, and one-way ANOVA, followed by the Tukey HSD post hoc test which was used to compare the means of the metabolite concentrations among the four groups, if ANOVA was significant. P values <0.05 were considered as statistically significant.

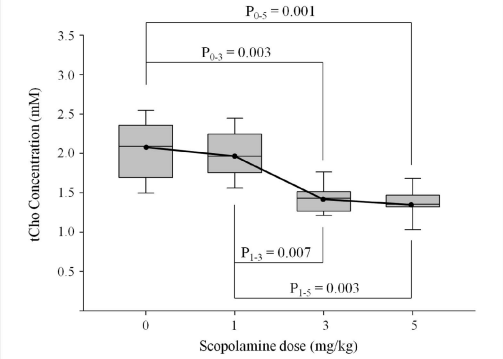

Fig.1 At 30min after injection, the level of tCho decreased at 3 mg/kg or more: bars not sharing a letter designation are significantly different from each other, i.e. $p < 0.05$.

RESULTS

Absolute quantification was performed with 8 metabolites (Gln, Glu, GSH, Ins, Tau, tCho, tNAA and tCr) which were 20% CRLBs or less. At 30 min after SCP injection, the levels of tCho at 3 and 5 mg/kg SCP dose were significantly reduced as comparing with 0 and 1 mg/kg: $F(3,28) = 10.231$, $P = 0.0001$ (Fig.1). Fig.2 shows the concentrations of 8 metabolites in each group at 30 min and 72 hour after SCP injection. In the 3 and 5 mg/kg dose injection groups, the level of tCho was decreased at 30 min after injection and recovered at 72 hours post injection. (3 mg/kg dose: P=0.024, 5 mg/kg dose: P=0.015). On the other hand, there was no statistically significant metabolic change observed in the 0 and 1 mg/kg dose groups.

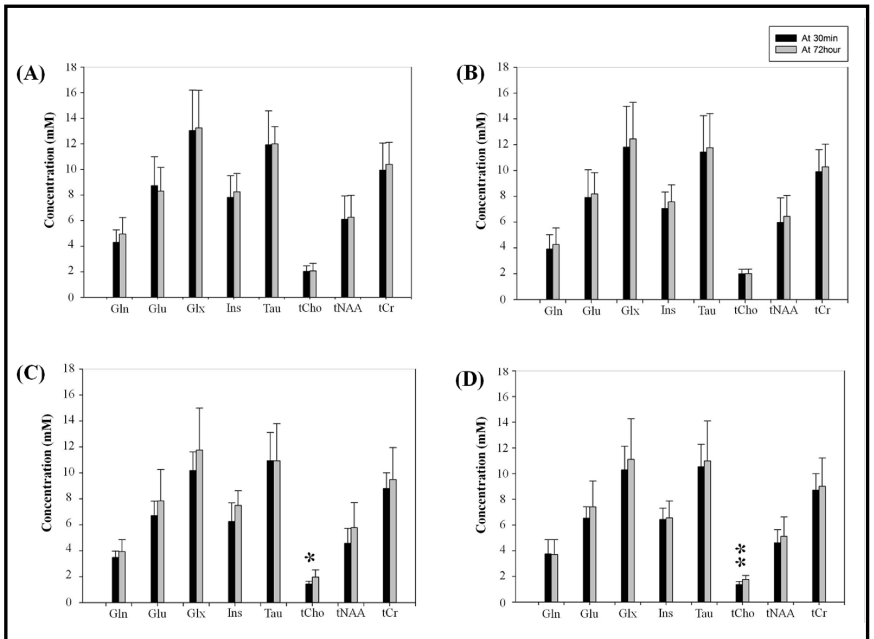

Fig.2 The neurochemical profiles of (a) 0, (b) 1, (c) 3, and (d) 5 (mg/kg) SCP induced changes in mouse brain (mean ± SD). In the 0 and 1 mg/kg SCP dose groups, there was no change between 30 min and 72 hours after injection. At 3 and 5 mg/kg SCP dose, the level of tCho level recovered after 72 hours. *P=0.024, **P=0.015.

DISCUSSION

The present study is the first in vivo assessment of the dose- time-dependent effects of SCP administration on the levels of cerebral metabolites. This work demonstrates that an injection of 3 (mg/kg) SCP or more reduces the level of tCho in the brain for short periods and the level recovers after 72 hours. Our results are in good agreement with previous published reports are showed that SCP reduced the level of ACh in the brain for about 2 hours [3]**.** It is known that *in vivo* NMR spectroscopy is able to detect the level of ACh in the brain [3, 4]. Finally, we suggest our findings may provide important evidence for altered cholinergic activity in the SCP induced model for memory impairment. A better understanding of interplay between cholinergic and glutamatergic neurotransmission by ^{1}H MRS may lead to the development of novel therapeutic approaches for treating memory deficits, as well as leading to new insights into the mechanisms of memory and learning and their deficits.

REFERENCES

[1] Stone W. et al. Psychopharm 1988;96:417-420. [2] Blake M. et al. Neurobiol Learn Mem 2012;98:112-121. [3] Pazzagli A. and Pepeu G. Int J Neuropharmacol 1964;4:291-299. [4] Stadler H. and Fuldner H. Nature 1980;286:293-294. [5] Wang X. et al. Neurochem Res 2008;33:814-819.