Neurochemical Changes Observed by In Vivo Proton Magnetic Resonance Spectroscopy in the Mouse Brain Postadministration of Scopolamine

Dong-Cheol Woo, PhD, Robert E. Lenkinski, PhD

Rationale and Objectives: This study is aimed at investigating neurochemical changes in scopolamine (SCP)–induced memory impairment using spatially localized in vivo magnetic resonance spectroscopy (MRS) of the hippocampus.

Materials and Methods: Four groups of mice (eight mice per group) were scanned after the injection of different SCP doses: 0, 1, 3, and 5 mg/kg (intraperitoneally). All the animals received ^{1}H MRS of their hippocampus at two time intervals: 30 minutes and 72 hours after SCP injection.

Results: This work demonstrated that the doses of 3 mg/kg SCP or higher reduce the concentration of total choline–containing compounds, and these levels returned to baseline after 72 hours. These results are consistent with observations made by others using more invasive brain dialysis approaches. The levels of glutamate and glutamic compounds (glutamate + glutamine) were slightly changed at 3 and 5 mg/kg SCP dose, but the differences were not statistically significant ($P > .05$). These findings suggest that SCP produces transient, in vivo measurable alterations in the cholinergic system in the hippocampus.

Conclusions: On this basis, we conclude that in vivo MRS is a feasible noninvasive method to probe aspects of the alterations induced by SCP in the cholinergic neurotransmission pathways in both animal models and human studies of memory impairment.

Key Words: ^{1}H MR spectroscopy; scopolamine; memory impairment; cholinergic system.

Cholinergic deficiency in human brain is the most severe and consistent biochemical change in mild cognitive impairment (MCI) and Alzheimer disease (AD) (1–3). This is seen as reduced levels of acetylcholine (ACh), choline acetyltransferase, and acetylcholinesterase reported in both necropsy brain samples and cerebrospinal fluid. In general, animal models of memory impairment have been used to understand the molecular basis of cognitive decline and identify therapeutic targets. Scopolamine (SCP), which is known as a muscarinic cholinergic receptor antagonist, has been used to induce memory deficit/amnesia such as is present in MCI and AD. SCP inhibits central cholinergic neuronal activity and impairs learning and short-term memory (4–7). Numerous studies have tried to find treatments that attenuate the effects of SCP-induced memory impairment (8–12). Several studies reported that the mechanism of memory impairment by cholinergic deficits may be through the modulation of the cerebrovasculature (13–15) and decreased blood flow and blood volume may induce rapid cognitive decline (16). Although the precise molecular details and the influence of SCP on neurometabolic pathways have not been completely elucidated, SCP has been used widely to produce animal models of memory impairment.

The development of spatially localized in vivo proton magnetic resonance spectroscopy (^{1}H MRS) of the brain has led to both mechanistic studies of brain metabolism in animal models of disease and numerous clinical studies of human diseases and their progression (17,18). ^{1}H MRS is well suited for the study of cognitive disorders, because it is noninvasive, reproducible, and can sample the in vivo metabolism of many brain regions. At present, ~20 compounds can be detected on 3T and 7T whole body magnetic resonance (MR) scanners. Some of these compounds are neurotransmitters (19,20). In particular, a resonance reflecting a composite peak representing total choline–containing compounds (tCho) is one of important metabolites in ^{1}H MRS studies of memory

Acad Radiol 2014; 21:1072–1077

From the Asan Institute for Life Sciences, Asan Medical Center, Songpa-gu, Seoul, South Korea (D.-C.W.) and Department of Radiology, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9061 (D.-C.W., R.E.L.). Received December 16, 2013; accepted April 14, 2014. **Address correspondence to:** R.E.L. e-mail: Robert.Lenkinski@UTsouthwestern.edu

http://dx.doi.org/10.1016/j.acra.2014.04.003

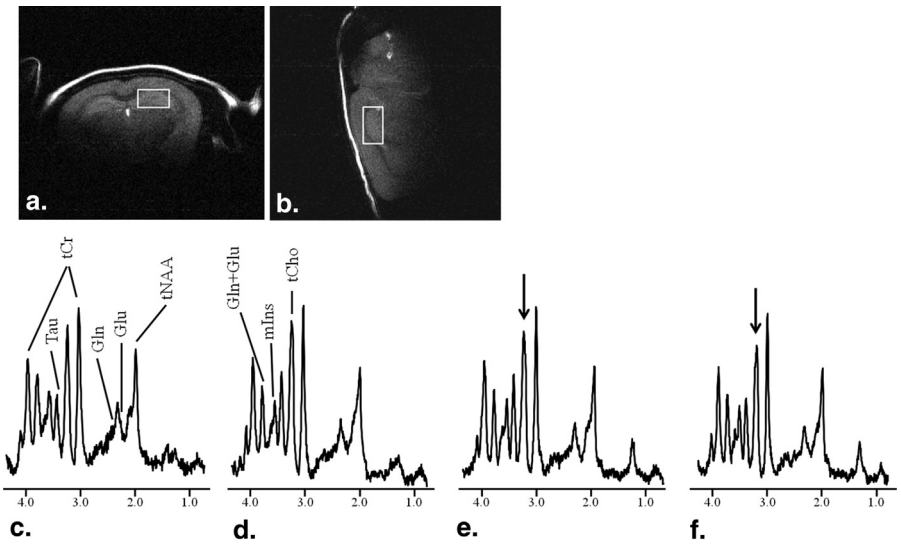

Figure 1. Localized hippocampal region: **(a)** axial and **(b)** sagittal view (voxel size: 1.5 × 2.0 × 2.5 mm) and magnetic resonance spectra at 30 minutes after **(c)** 0, **(d)** 1, **(e)** 3, and **(f)** 5 mg/kg scopolamine dose.

impairment. Some studies have reported that total choline signal in ^{1}H MRS has been changed in MCI and AD patients (21,22). This total choline peak is made up of ACh, glycerophosphocholine (GPC), phosphocholine (PC), and free choline (23,24).

Until now, studies of the effect of SCP on the brain, primarily memory impairment by cholinergic deficits, have been evaluated by invasive methods, such as high-performance liquid chromatography, and behavioral tests (4,25–29). To date, there have been no reported noninvasive in vivo ^{1}H MRS studies aimed at determining any metabolic changes in the brain as a result of SCP administration. In this study, we report that noninvasive in vivo ^{1}H MRS can detect metabolic changes in SCP-induced memory impairment in the brain of mice. We also show time- and dose-dependent neurochemical changes induced by the administration of SCP in this murine model.

MATERIALS AND METHODS

Animals and Drugs

Adult male Swiss albino mice (12–16 weeks) weighing 25–30 g (n = 32) were housed (four mice per cage) on sawdust and kept on a 12/12 hours light–dark cycle, with free access to food and water throughout the experiment. All the procedures were performed under an approved protocol by the Institutional Animal Care and Use Committee of University of Texas Southwestern Medical Center. All efforts were made to minimize the number of animals used and their distress.

Scopolamine hydrobromide was obtained from the Sigma–Aldrich Company, dissolved in 0.9% normal saline (0.2 mL) and administered by intraperitoneal injection. Mice were assigned to four groups according to doses: SCP 0, 1, 3, and 5 mg/kg. All the mice received ^{1}H MRS as soon as they were anesthetized at 30 minutes and 72 hours after SCP injection.

Proton Magnetic Resonance Imaging/Magnetic Resonance Spectroscopy

All the mice were anesthetized by spontaneous inhalation of 1.5%–2% isoflurane mixed in air and delivered via a mask using an anesthesia unit to minimize the motion of the mouse during MR scanning. The mice were placed in the handling system in prone position, and a homemade surface coil (1-cm diameter) was used to acquire signals. The animals breathed freely during an hour of total MR acquisition and were monitored for changes in the respiratory rate to adjust the concentration of anesthetic.

All the MR studies were carried out in a horizontal 9.4T/16 cm magnet (Agilent, Palo Alto, CA), with 400 mT/m gradient sets. For localization and identification of the anatomic landmarks, T2-weighted MR images (fast spin echo [FSE]) were acquired: repetition time (TR)/ echo time (TE) = 3000/10 milliseconds, matrix size = 256 × 256, slice thickness = 1.0 mm, NEX = 2, and 12 slices. The volume of interest (VOI) was adjusted to include the left hippocampal region. The size of VOI for MRS was 7.5 μL (1.5 × 2.0 × 2.5 mm^{3}). A point-resolved spectroscopy sequence was used to localize the VOIs: TR/TE = 3000/19 milliseconds, average = 512, complex data points = 4096, spectral width = 4000 Hz. VAPOR-optimized pulse power and timing was used to suppress the water signal. For quantification, the unsuppressed water signals were also obtained (average = 4). All the procedures for MR scanning (MRI + MRS) were finished within 1 hour.

Quantification

All the in vivo ^{1}H MR spectra were processed with the linear combination analysis method (LCModel ver. 6.0; Los Angeles, CA) to calculate metabolite concentrations from a fit to the experimental spectrum, based on simulated basis set. The following brain metabolites in the basis set were included: