Chronic citicoline increases phosphodiesters in the brains of healthy older subjects: an in vivo phosphorus magnetic resonance spectroscopy study

S. M. Babb [1, 2], L. L. Wald [3], B. M. Cohen [1], R. A. Villafuerte [1], S. A. Gruber [1], D. A. Yurgelun-Todd [1] and P. F. Renshaw [1]

(1) Brain Imaging Center, McLean Hospital, 115 Mill St., Belmont, MA 02478, USA, (2) Consolidated Department of Psychiatry, Harvard Medical School, Boston, Massachusetts, USA, (3) Department of Radiology, MGH-NMR Center, Massachusetts General Hospital, 49 13th St, Charlestown, MA 02129, USA

Abstract

Rationale: Phosphatidylcholine (PtdCho) in brain cell membranes decreases with age. Evidence from both animal and in vitro studies indicates that CDP-choline (citicoline) administration may increase PtdCho synthesis and might reverse PtdCho loss. Objectives: We investigated whether oral citicoline can increase PtdCho synthesis in the brains of older subjects by measuring levels of phosphorus-containing metabolites using proton-decoupled phosphorus magnetic resonance spectroscopy (^{31}P-MRS) before and after citicoline treatment. Methods: All subjects took 500 mg citicoline once orally each day for 6 weeks, then took either citicoline or placebo once orally per day for a second 6-week period. Subjects underwent a ^{31}P-MRS scan at baseline and following 6 and 12 weeks of treatment. Results: Treatment with citicoline for 6 weeks was associated with a 7.3% increase from baseline levels in brain phosphodiesters (P=0.008), including an 11.6% increase in glycerophosphoethanolamine (P=0.002) and a 5.1% increase in glycerophosphocholine (P=0.137). Subjects who continued to take citicoline for the second 6-week period did not show significant additional increases in the levels of these metabolites. No changes were seen in other phosphorus-containing metabolites. There was a correlation between improvement on the California Verbal Learning Test and increase in phosphodiesters. Conclusions: The increases in phosphodiesters seen in this study indicate that phospholipid synthesis and turnover were stimulated by 6 weeks of oral citicoline. These results in humans support previous in vitro and animal studies and suggest that the administration of oral citicoline may be of use in reversing age-related changes in the brain.

Introduction

A decline in cognitive function is commonly seen with age and may be associated with biochemical and metabolic changes occurring in the central nervous system[1-3]. Many of these changes may be reflected in levels of phosphorus-containing metabolites in the brain, which can be detected by phosphorus magnetic resonance spectroscopy (^{31}P-MRS). Specifically, ^{31}P-MRS can identify phosphomonoesters (PME) and phosphodiesters (PDE), which are integral to structural membrane synthesis and are also involved in the biosynthetic pathway of the neurotransmitter acetylcholine (ACh).

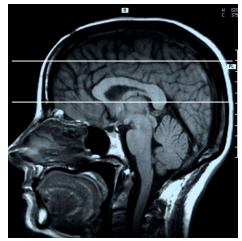
Levels of ACh are lower in the brains of older persons and especially those with Alzheimer's disease, and it is thought that this may lead to a breakdown of cell membrane lipids to supply the needed precursors for ACh synthesis[4,5]. A reduction in phospholipid phosphatidylcholine (PtdCho) has even been observed in normal aging[6,8], and this decrease may affect the function of receptors, ion channels, and enzymes that span or are bound to the cell membrane[9,10].

Consequently, a drug that prevents the age-related breakdown of membrane phospholipids might be therapeutically useful. CDP-choline (citicoline) appears to be such a compound, as it provides an exogenous source of precursors and a catalyst for the synthesis of both ACh and membrane phospholipids[11].

In the current study, we attempted to provide more direct evidence that oral citicoline can increase PtdCho synthesis in the brains of older subjects by measuring levels of phosphorus-containing metabolites, including the anabolites (PME) and catabolites (PDE) of PtdCho, as well as the broad phospholipid resonance, using proton decoupled ^{31}P-MRS.

Methods

All subjects (7 males, 12 females) were volunteers recruited from the local community and screened by medical history, physical examination and laboratory tests to be free of serious medical, neurological or psychiatric illness. All subjects were Caucasian, with a mean age of 70.3±5.7 years.



Subjects underwent a baseline proton decoupled ^{31}P-MRS scan and neuropsychological testing before taking 500 mg citicoline orally per day for 6 weeks. As there was a wide variation in body weight of subjects, the citicoline dose ranged from 5.1 to 13.1 mg/kg/day. At the end of the first 6-week treatment period, subjects returned for a second ^{31}P-MRS scan. Subjects then took either citicoline or placebo (double blind, randomly assigned) once orally per day for a second 6-week period. At the completion of the trial (week 12), subjects returned for neuropsychological testing, as before, and a third ^{31}P-MRS scan.

Fig. 2 Treatment with citicoline for 6 weeks was associated with a 7.3% increase from baseline levels in brain phosphodiesters (P=0.008), including an 11.6% increase in glycerophosphoethanolamine (P=0.002) and a 5.1% increase in glycerophosphocholine (P=0.137).

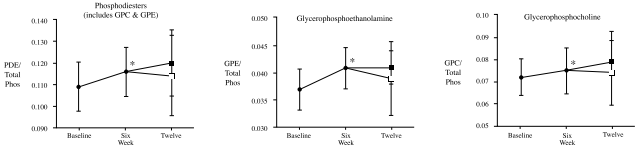
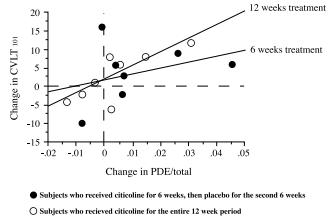


Fig. 3 Correlation between change in the California Verbal Learning test (total recall) and change in PDE/total phosphorus between the 12-week time point and baseline.



Results

Treatment with citicoline for 6 weeks was associated with an average 7.3% increase in brain PDE (including both glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE)) (mean difference=0.007, P=0.008, paired t test, two-tailed) from the baseline levels, including an average 11.6% increase in GPE (mean difference=0.004, P=0.002) and a statistically insignificant average increase of 5.1% in GPC (mean difference=0.003, P=0.137) (Fig. 2).

Although slight increases above baseline were seen in PDE, GPE and GPC at the 12-week measurement, only the change in PDE was statistically significantly different from baseline at the P=0.05 level (paired t tests, two-tailed) (PDE increased an average of 6.5% (mean difference=0.007, P=0.070), GPE an average of 6.6% (mean difference=0.002, P=0.062) and GPC an average of 6.6% (mean difference=0.005, P=0.118). From weeks 6 to 12, half of the subjects continued to receive citicoline and half received placebo. Subjects who continued to take citicoline for the entire 12 weeks did not have significantly higher metabolite levels than subjects who had only taken citicoline for the first 6 weeks, and then placebo from week 6 until week 12. Paired t tests did not show any further changes in PDE between weeks 6 and 12 (6-week treatment, P=0.740; 12-week treatment P=0.882), GPE (6-week treatment, P=0.178; 12-week treatment P=0.478) or GPC (6-week treatment, P=0.515; 12-week treatment P=0.962) (Fig. 2).

Statistically significant correlations emerged between improvement in verbal learning, as measured by total recall on the California Verbal Learning Test (CVLT) (the total number of words correctly recalled across all five initial CVLT trials), and increases in metabolite ratios. A greater improvement in performance on CVLT total recall was associated with a greater increase in PDE, GPC and GPE. All subjects underwent neurocognitive testing at baseline and 12 weeks.

Figure 3 shows the relationship between change in CVLT total recall and change in PDE between week 12 and baseline for both treatment groups. A statistically significant correlation was detected in the subjects who received citicoline for the entire 12-week period between the change in CVLT total recall and the change in the metabolite ratios PDE/total (P=0.014, r=0.775) and GPE/total (P=0.023, r=0.739); but there was no statistically significant correlation between CVLT total recall and PDE/total (P=0.417, r=0.335) or GPE/total (P=0.399, r=0.348) for the 6-week treatment group or in GPC/total for either treatment group (12 weeks, P=0.060, r=0.647); (6 weeks, P=0.479, r=0.295). In addition, there was slightly greater improvement on scores for CVLT total recall in the group that received citicoline for 12 weeks than in the 6-week treatment group (increased score improvement 3.2 vs 2.6 points). However, this performance difference between groups with different treatment lengths did not reach statistical significance (P=0.84, ANOVA), nor were the CVLT total scores significantly higher at 12 weeks than at baseline (12-week treatment, P=0.16, 6-week treatment P=0.35, all subjects P=0.09: paired t tests).

Discussion

Measurable increases in PDE, compounds involved in phospholipid biosynthesis and breakdown, were induced by oral treatment with citicoline. These increases in PDE may indicate that phospholipid synthesis and turnover were stimulated by 6 weeks of oral citicoline, which may increase PtdCho, PtdEtn and PtdSer incorporation into brain cell membranes.

It is interesting that there was a correlation between improvement in CVLT total scores and increased PDE/total brain phosphorus levels. A decline in learning and memory is commonly observed with normal aging, and has been hypothesized to be linked with changes in brain cholinergic mechanisms . A recent comprehensive review concluded that citicoline does have a beneficial effect on memory function, probably by providing precursors to promote the synthesis of both ACh and membrane phospholipids.

This study provides the first in vivo data in humans to support the many in vitro and animal studies showing increased PtdCho synthesis as a consequence of citicoline administration. The correlation between increased PDE concentrations and improved verbal learning in healthy older subjects suggests that the administration of oral citicoline may be of use in reversing age-related cognitive changes.

Poster sponsored by Kyowa Hakko

1. Bartus, R.T., et al., *The cholinergic hypothesis of geriatric memory dysfunction.* Science, 1982. **217**(4558): p. 408-14.
2. Pepeu, G. and L. Giovannelli, *The central cholinergic system during aging,* in *Progress in brain research*, F. Bloom, Editor. 1984, Elsevier: Amsterdam. p. 67-71.
3. Hoyer, S., *Oxidative metabolism deficiencies in brains of patients with Alzheimer's disease.* Acta Neurol Scand Suppl, 1996. **165**: p. 18-24.
4. Wurtman, R.J., J.K. Blusztajn, and J.C. Maire, *"Autocannibalism" of choline-containing membrane phospholipids in the pathogenesis of Alzheimer's disease: a hypothesis.* Neurochem Int, 1985. **7**: p. 369-372.
5. Maire, J.C. and R.J. Wurtman, *Choline production from choline-containing phospholipids: a hypothetical role in Alzheimer's disease and aging.* Prog Neuropsychopharmacol Biol Psychiatry, 1984. **8**(4-6): p. 637-42.
6. Rouser, G. and A. Yamamoto, *Curvilinear regression course of human brain lipid composition changes with age.* Lipids, 1968. **3**: p. 284-287.
7. Jellinger, K., et al., *Changes of phospholipids in Alzheimer's disease brain,* in *Alzheimer's disease: advances in clinical and basic research*, B. Corain, et al., Editors. 1993, Wiley: Chichester. p. 315-323.
8. Roth, G.S., J.A. Joseph, and R.P. Mason, *Membrane alterations as causes of impaired signal transduction in Alzheimer's disease and aging.* Trends Neurosci, 1995. **18**(8): p. 203-6.
9. Loh, H.H. and P.Y. Law, *The role of membrane lipids in receptor mechanisms.* Annu Rev Pharmacol Toxicol, 1980. **20**: p. 201-34.
10. Henis, Y.I., *Lateral mobility measurement of cell surface components: applications for molecular pharmacology.* Trends Pharmacol Sci, 1989. **10**(3): p. 95-8.
11. Secades, J.J. and G. Frontera, *CDP-choline: pharmacological and clinical review.* Methods Find Exp Clin Pharmacol, 1995. **17 Suppl B**: p. 1-54.
12. Fioravanti, M. and M. Yanagi, *Cytidinediphosphocholine (CDP choline) for cognitive and behavioural disturbances associated with chronic cerebral disorders in the elderly.* Cochrane Database Syst Rev, 2000(4): p. CD000269.