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Differential effect of CDP-choline on brain cytosolic choline levels in younger and older subjects as measured by proton magnetic resonance spectroscopy

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Abstract Phosphatidylcholine (PtdCho), which is essential for membrane integrity and repair, is reduced in brain cell membranes with age. Evidence from both animal and in vitro studies indicates that cytidine 5'-diphosphate choline (CDP-choline) can increase the synthesis of PtdCho; however, the effect of CDP-choline on brain choline metabolism has not previously been studied in human subjects. In this study, in vivo proton magnetic resonance spectroscopy ((1H-MRS)) was used to measure brain levels of cytosolic, choline-containing compounds before and after single oral doses of CDP-choline. Three hours after dosing, plasma choline increased similarly in younger (mean age 25 years) and older subjects (mean age 59 years). However, while the choline resonance in brain increased by 18% on average in younger subjects, it decreased by almost 6% in older subjects \( P = 0.028 \). These results may be explained by a previously observed decrease in brain choline uptake, but not cytidine uptake, in older subjects. Additional intracellular cytidine following the administration of CDP-choline should lead to the increased incorporation of choline already present in brain into membrane PtdCho, which is not MRS-visible, consequently lowering the brain choline resonance below that of pre-treatment values. These results suggest that the cytidine moiety of CDP-choline stimulates phosphatidylcholine synthesis in human brain cell membranes in older subjects.

Key words 5'-Cytidine diphosphate choline · Choline · Phosphatidylcholine · Cytidine · Aging · Brain · Magnetic resonance spectroscopy

Introduction

Declines in cognitive function and neuronal plasticity with age have been associated with specific changes occurring in the central nervous system, including decreases in the functioning of cholinergic neurons in the brain (Bartus et al. 1982; Pepeu and Giovannelli 1994) and alterations in cell membrane composition, notably a reduction of choline-containing phospholipids (Rouser and Yamamoto 1968; Jellinger et al. 1993; Roth et al. 1995). Moreover, acceleration of these age-related changes may be associated with the development of neurodegenerative disorders, including dementia.

These degenerative changes may be due, at least in part, by age-related alterations in the transport or metabolism of choline in brain. Although choline is a precursor of both the neurotransmitter acetylcholine (ACh), as well as the phospholipids phosphatidylcholine (PtdCho) and sphingomyelin, essential structural components of cell membranes, very little choline is synthesized in brain (Tucek 1984; Wurtman 1992; Scremin and Jenden 1993). While choline can be synthesized by the liver (Hicks et al. 1982), brain choline is largely obtained through dietary intake (Zeisel 1992; Klein et al. 1993), and transported across the blood-brain barrier by facilitated diffusion (Cornford et al. 1978; Millington and Wurtman 1982; Klein et al. 1991; Löffelholz et al. 1993). An animal study (Mooradian 1988), and more recently, human studies (Cohen et al. 1994, 1995) have shown that choline uptake into brain is reduced in older subjects. This reduction in choline uptake may contribute to the decrease in membrane PtdCho levels (Rouser and Yamamoto 1968; Jellinger
et al. 1993; Roth et al. 1995) and cholinergic function observed in the brain with age (Bartus et al. 1982; Pepeu and Giovannelli 1994).

Many treatments to improve cognitive function in normal aging or Alzheimer's disease (AD) have focused on increasing acetylcholine (ACh) levels in the brain. These treatments have shown only minimal success (Bartus 1990), perhaps because the availability of choline in brain decreases with age. An alternative therapeutic strategy involves the correction of age-related changes in the structure of brain cell membranes by increasing the incorporation of cytosolic choline into membrane phospholipids. A candidate treatment for this purpose is 5'-cytidine diphosphate choline or CDP-choline, an endogenous compound which is a key intermediate in the biosynthesis of phosphatidylcholine (PtdCho) from choline (Kennedy and Weiss 1956) (Fig. 1). CDP-choline has been used clinically to treat patients with head trauma, cerebral vascular disease and various cognitive disorders (for review see Secades and Frontera 1995). The clinical efficacy of CDP-choline is presumably due to its ability to increase PtdCho synthesis in injured brain (Galletti et al. 1991). It may have a similar effect in aging brain, as CDP-choline has been reported to increase PtdCho levels in membrane preparations from 12-month-old mice (Lopez G-Coviella et al. 1988). Additionally, oral CDP-choline increased brain PtdCho by 23% after 42 days of treatment and by 30% after 90 days of treatment in 12-month-old rats (Agut et al. 1993).

To date, studies on brain phospholipid and choline metabolism in living human subjects have not been performed. However, such studies are made possible by the recent development of in vivo proton magnetic resonance spectroscopy (¹H MRS), which can detect cytosolic, choline-containing compounds in brain (Petroff et al. 1988; Frahm et al. 1989). It is important to note however, that compounds such as PtdCho, which are immobilized in cell membranes, are largely invisible to MRS (Miller 1991).

Since ¹H-MRS is non-invasive and free of the requirement for ionizing radiation, multiple studies may safely be performed on the same subject. Thus, ¹H-MRS provides a means directly to assess the effects of drugs on brain choline metabolism (Stoll et al. 1995).

In this study, in vivo ¹H-MRS was used to measure choline-containing compounds in human brain to determine whether single oral doses of CDP-choline
would alter brain choline metabolism in younger and older adults.

**Materials and methods**

All subjects were volunteers chosen from lists available to McLean Hospital from the Clinical Research Center at Massachusetts Institute of Technology and through advertisement in a local newspaper. Subjects were screened by medical history, physical examination and laboratory tests to be free of serious medical, neurological or psychiatric illness. All of the older subjects were Caucasian, two of the younger subjects were Hispanic and one was Asian. Priming subjects were six males with a mean age of 23 ± 3 years; older subjects were four males and two females with a mean age of 59 ± 3 years. No younger subjects were taking prescription or non-prescription drugs. One of the older subjects was taking triamterene for high blood pressure. Five of the older adults took no regular medication. No subject was receiving a medication known to affect gastrointestinal absorption, in general, or the absorption and metabolism of choline in particular (Kang et al. 1990).

Following screening, subjects participated on 3 study days, each separated by at least 1 week. Before each study, subjects were asked to fast overnight, drinking only water. A baseline MR scan including proton spectroscopy was performed. Following the scan, one tube (5 ml) of blood was drawn and immediately centrifuged for 10 min to separate plasma, which was then frozen on dry ice and stored in a −70°C freezer until assayed for choline and PtdCho.

Plasma choline and PtdCho were measured by gas chromatography/mass spectrometry using the method of Zeisel and daCosta (1990) by researchers blind to the origin of the specimen. Subjects were given 0.5 g, 2.0 g and 4.0 g CDP-choline in a random order over the 3 study days, administered in a double blind manner. After a 3-h period, MR spectroscopy and blood drawing were repeated as before. The 3-h time point was chosen from the results of animal experiments which indicated that the choline moiety of exogenously administered CDP-choline is already being utilized in brain phospholipid biosynthesis at this point after dosing (De Rosa et al. 1985).

The protocol was approved by the McLean Hospital Institutional Review Board, and all subjects gave written informed consent for these procedures.

**MRS procedure**

MR images and spectra were acquired using a 1.5T Signa whole body scanner (GE Medical Systems, Milwaukee). An 8 cm$^3$ voxel centered on the head of the caudate and the putamen was determined from coronal, T1-weighted, 3 mm contiguous scout images. The basal ganglia were chosen as a representative site because they contain a mixture of white and gray matter and cholinergic as well as other neurons. Voxel placement was chosen to contain no or minimal (<2%) CSF. A modified STEAM pulse sequence was used to acquire the $^1$H-MR spectra using TR = 2 s, TE = 30 ms, 1024 data points, and 2500 Hz spectral width (Webb et al. 1994). The total data acquisition time was just over 8 min as 256 transients were averaged. Spectra were zero-filled to 2048 data points and filtered with an exponential function to produce a line broadening of 1 Hz after Fourier transformation. The transformed spectra were baseline corrected with a spline function to compensate for residual water signal. Following phase correction, peaks were fit by two independent raters to Gaussian lineshapes using a Marquardt algorithm.

Two major resonance signals were examined, choline (Cho) and creatine (Cr). Several cytosolic choline containing compounds in the brain (Frahm et al. 1989; Miller 1991), mainly phosphocholine and glycerophosphocholine, as well as choline, acetylcholine, and CDP-choline, contribute to the brain Cho resonance. Phosphatidylcholine (PtdCho) is immobilized in cell membranes and is largely invisible to MRS (Miller 1991). The Cr resonance is composed of creatine and phosphocreatine and is often used as an internal standard, because this signal appears stable over time and across a range of physiological states within an individual subject (Petroff 1988; Petroff et al. 1989). Relative concentrations of brain Cho were determined using the ratio of the Cho resonance intensity to the Cr resonance intensity (Cho/ Cr).

All MR spectra were analyzed independently by two researchers blind to the origin of the data. The mean of their results for each spectrum was used in the data analysis. The intraclass correlation coefficient for measurements of the Cho/Cr ratio by the two raters was $r = 0.84$ (n = 72 spectra). For the statistical analysis of the data, analysis of variance (ANOVA) was performed. Statistical significance was considered to be $P \leq 0.05$.

**Results**

Plasma choline levels before and after CDP-choline treatment for each dose and in each age group are shown in Table 1. Plasma choline tended to be higher both before and after dosing in the older subjects relative to the younger subjects, and in some trials, this group difference reached statistical significance. When data from all trials are combined for each age group, plasma choline was found to be significantly higher in the older than in the younger subjects both at baseline ($P = 0.002$), and post-treatment ($P < 0.0001$). Changes in plasma choline due to CDP-choline administration (post-treatment minus baseline) are shown in Fig. 2. Plasma choline increased in each case after CDP-choline treatment. However, because of relatively large individual differences, the only statistically significant difference within each age group between baseline and post-treatment values is at the 2000 mg dose in the older subjects ($P = 0.002$). The magnitude of these

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<th>Dose</th>
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<td><strong>Young</strong></td>
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<td>Young</td>
<td>7.6 ± 0.63</td>
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<tr>
<td>Old</td>
<td>9.8 ± 0.68</td>
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| **4000 mg** | | | | | | |
| Baseline | Post | Baseline | Post | Baseline | Post | |
| Old | 7.7 ± 0.35 | 8.7 ± 0.38 | 9.3 ± 0.33 | 11.0 ± 0.32 | | |
increase were not significantly different between old and young at any dose or when all doses were combined (P = 0.11, repeated measures ANOVA). Within each age group, there was no statistically significant difference between doses in the magnitude of the increase in plasma choline (P = 0.72, old; P = 0.43, young). Also, there was no significant correlation between dose and increase in plasma choline, even though there appeared to be a trend for plasma choline to increase with CDP-choline dose in the young subjects (r = 0.37; P = 0.13).

Plasma PtdCho levels before and after CDP-choline treatment are shown in Table 2. As with plasma choline levels, plasma PtdCho was higher in older subjects both at baseline and post-treatment and, in most cases, this difference was statistically significant. The changes (post-treatment minus baseline) in plasma PtdCho were small and there were no statistically significant differences between baseline and post-treatment values within either group at any dose, or between old and young at any dose or for all doses combined (P = 0.44, repeated measures ANOVA).

Brain Cho/Cr ratios before and after treatment with CDP-choline are shown in Table 3. Brain Cho/Cr tended to be higher before treatment, but lower after treatment, in the older compared to the younger subjects. Because of large interindividual variance, the only statistically significant difference between baseline and post-treatment values was at the 4000 mg dose in the older subjects (P = 0.03). Changes in brain Cho/Cr ratio due to CDP-choline administration (post-treatment minus baseline) are shown in Fig. 3. The changes in brain Cho/Cr ratio from all CDP-choline doses were analyzed using repeated measures ANOVA and the change in Cho/Cr in older subjects (an average decrease of 0.088 ± 0.050) and in younger subjects (an average increase of 0.136 ± 0.061) were significantly different (P = 0.017). Furthermore, the decrease of 0.088 in the brain Cho/Cr ratio in the older subjects is significantly different from zero change (t-test, P = 0.049), and the increase of 0.136 in the brain Cho/Cr ratio in the younger subjects is significantly different from zero change (t-test, P = 0.019). This is equivalent to a mean decrease of 5.8% in older subjects and a mean increase of 18.0% in younger subjects (P = 0.028, repeated measures ANOVA). This difference between older and younger subjects was not due to the presence of the two female subjects in the older group. The change in
an increase in plasma choline (Bligh 1953), increased (Hicks et al. 1982). As some disorders of the liver cause an age-related decline in liver enzyme function. Alternatively, it may be a consequence of altered dis-

the rates of choline synthesis and oxidation by the liver is not known. However, plasma choline concentration shown to be higher in older subjects than in younger subjects (Eckernäs and Aquilonius 1977; Hartford et al. 1984). The reason for this age-related difference is dependent upon dietary intake of choline, as well as the rates of choline synthesis and oxidation by the liver (Hicks et al. 1982). As some disorders of the liver cause an increase in plasma choline (Bligh 1953), increased plasma choline levels in older subjects may be due to an age-related decline in liver enzyme function. Alternatively, it may be a consequence of altered dis-

Discussion

All doses of CDP-choline in both groups of subjects tended to lead to increases in plasma choline levels. The magnitude of these increases was similar in older and younger subjects. However, as older subjects had a higher baseline level of plasma choline, they had higher post-treatment levels of plasma choline, as well. In humans, plasma choline levels have previously been shown to be higher in older subjects than in younger subjects (Eckernäs and Aquilonius 1977; Hartford et al. 1984). The reason for this age-related difference is not known. However, plasma choline concentration is dependent upon dietary intake of choline, as well as the rates of choline synthesis and oxidation by the liver (Hicks et al. 1982). As some disorders of the liver cause an increase in plasma choline (Bligh 1953), increased plasma choline levels in older subjects may be due to an age-related decline in liver enzyme function. Alternatively, it may be a consequence of altered dis-

tribution of choline between blood and tissues, including brain (Cohen et al. 1994, 1995).

Plasma PtdCho did not change substantially after CDP-choline treatment. However, as with plasma choline, plasma PtdCho was higher in the older subjects at baseline and, consequently, higher post-treatment than in the younger subjects.

In spite of similar increases in plasma choline after oral CDP-choline in older and younger subjects, the Cho/Cr ratio in brain changed quite differently in each group following treatment. In the older subjects, all doses of CDP-choline were followed by a decrease (4–16%) in the choline resonance, while in the younger subjects, all doses were followed by an increase (12–26%) in the choline resonance. Our recent studies have shown that the brain Cho/Cr ratio increases in both younger and older adults when choline is given alone, although this increase is greater on average in younger than in older subjects (Cohen et al. 1995). This maximum increase in brain Cho/Cr in younger subjects (26%) in the present study was less than that seen in younger subjects (60%) in our previous study in which subjects orally ingested 50 mg choline/kg body weight (Cohen et al. 1995). It should be noted that the highest dose of CDP-choline given in this study (4000 mg) was equivalent to 11–16 mg choline/kg body weight, depending on the size of the subject, accounting for the lower increase in the present study.

The opposite results on brain Cho/Cr seen in younger and older subjects may be explained by the effects of cytidine against a background of differences in choline uptake in younger and older subjects. Oral CDP-choline is metabolized to cytidine and choline in the periphery, with each moiety being separately taken into brain or other tissues (Galletti et al. 1991). While choline crosses the blood-brain barrier by substrate-specific, facilitated diffusion (Cornford et al. 1978; Millington and Wurtman 1982; Klein et al. 1991; Löffelholz et al. 1993), cytidine is apparently transported by a separate system of specific and saturable nucleoside transport processes, which are better characterized for nucleosides other than cytidine (Cornford and Oldendorf 1975; Kalaria and Harik 1986). It is not known if these two different transport processes are affected equally by aging. However, in vivo animal experiments with double-labeled CDP-choline suggest that after dosing, brain cytidine increases more rapidly and to a higher level than choline (Galletti et al. 1991) and, unlike choline, the cytidine moiety of CDP-choline might be taken up reasonably well by the brains of older adults. Our results are consistent with such a possibility.

Cytidine triphosphate (CTP) levels have been shown to be a critical regulator of PtdCho synthesis in cell culture systems (Whitehead et al. 1981) and 10 μM cytidine can increase cell membrane PtdCho levels in rat brain striatal slices even without additional choline (Savici and Wurtman 1995). Thus, in the older subjects,
it is likely that higher brain levels of cytidine lead to the increased incorporation of choline and phospholipid synthesis in brain. This study is the first to show evidence that CDP-choline can increase PtdCho synthesis in vivo as well as in human brain. Because the effects observed in this study may be due to the cytidine moiety of CDP-choline, studies on cytidine treatment alone would be valuable to define further its role in stimulating brain PtdCho synthesis in human subjects. The results of such studies should be interpreted with the knowledge that cytidine is used by cholinergic neurons both for PtdCho and ACh synthesis (Ulus et al. 1989). Therefore, administration of cytidine without concurrent administration of choline may channel available choline towards PtdCho synthesis and away from ACh synthesis. In some subjects, notably those with reductions in cholinergic neurotransmission produced by illness or medication, this diversion of choline might be associated with unwanted effects, at least transiently.

Nonetheless, cytidine has been shown to improve neuronal activity, as measured by EEG, in subjects with multi-infarct dementia (Gallai et al. 1991) and to stimulate PtdCho synthesis in rat striatal slices (Savci and Wurtman 1995). If cytidine can increase PtdCho synthesis in human brain cell membranes, it may be an effective treatment for neurological diseases and injuries resulting in membrane deficits. Also, age-related reductions in brain PtdCho with age may contribute to a decrease in the fluidity of cell membranes which may be associated with changes in the functioning of transmembrane receptor molecules and ion transport channels (Grinni et al. 1997; Cohen and Zubenko 1985; Miller 1990). By reversing such changes, CDP-choline or cytidine alone may help in preventing the brain membrane PtdCho reductions seen in normal aging and, thereby, may help in maintaining cognitive function in old age.

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References


Bligh J (1953) The role of the liver and the kidneys in the maintenance of the level of free choline in plasma. J Physiol (London) 120: 53-62


