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ORIGINAL INVESTIGATION

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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 (^{1}H-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.

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

In this study, in vivo ^{1}H-MRS was used to measure choline-containing compounds in human brain to determine whether single oral doses of CDP-choline

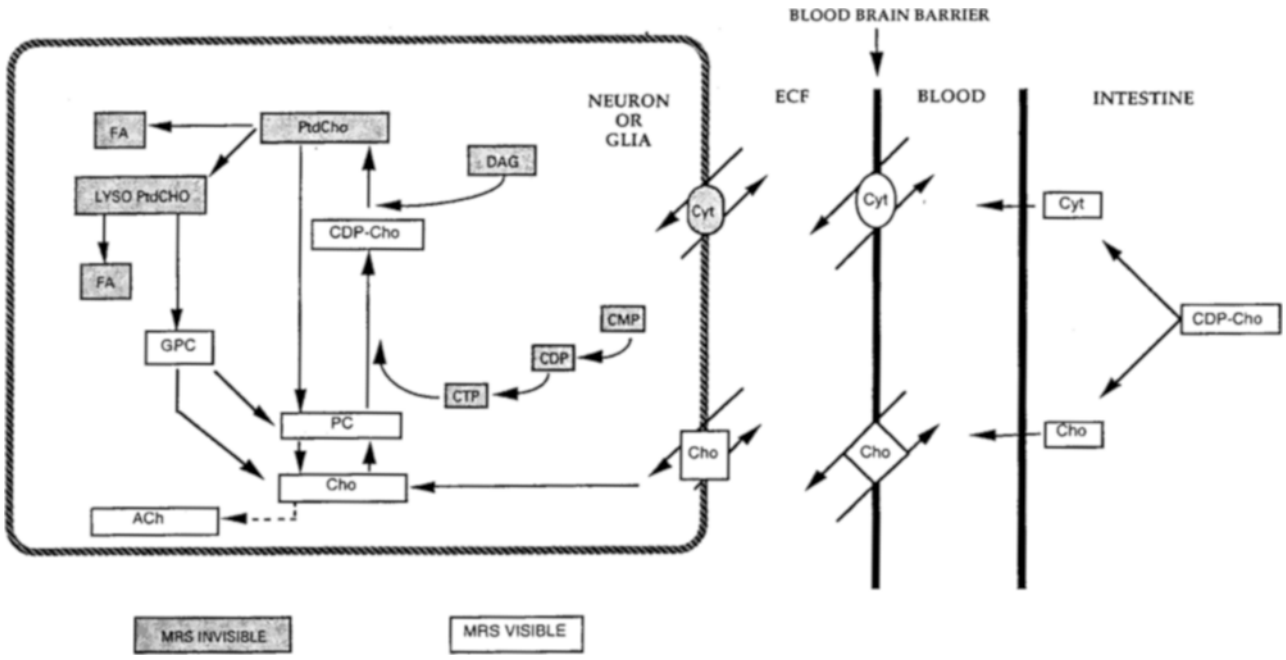

Fig. 1 Transport and metabolism of CDP Choline. When taken orally, CDP-choline is completely metabolized to cytidine (*Cyt*) and choline (*Cho*) in the intestine (Yashima et al. 1975). These moieties enter blood and are taken up into brain cells through separate mechanisms (Galletti et al. 1991). Once in the cell, cytidine is converted sequentially to the cytidine nucleotides cytidine monophosphate (*CMP*), cytidine diphosphate (*CDP*), and cytidine triphosphate (*CTP*) (Kennedy and Weiss 1956; Plagemann 1971). Studies in rats have shown that choline is rapidly taken up by the brain and phosphorylated to form phosphocholine (*PC*) within 10–30 min after IP injection of ^{3}H-choline (Klein et al. 1992). PC reacts with CTP to generate CDP-choline; this reaction, catalyzed by CTP:phosphocholine cytidylyltransferase (EC 2.7.7.15), is the rate-limiting step in the production of phosphatidylcholine (*PtdCho*) (Pelech and Vance 1984). CDP-choline rapidly combines with diacylglycerol (*DAG*) to yield PtdCho (Kennedy and Weiss 1956; Pelech and Vance 1984). Labeled PtdCho starts to appear in the brain at 30 min post-IP injection of labeled choline, increases greatly at 2 h post-injection, and increases further at 24 h, with concomitant loss of label from PC (Klein et al. 1992). Compounds in *shaded boxes* are invisible by MRS. *CDP-Cho* cytidine diphosphate choline, *LYSO PtdCho* lysophosphatidylcholine, *FA* fatty acid, *ACh* acetylcholine

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. Young subjects were six males with a mean age of 25 ± 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 adults 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

Table 1 Plasma choline levels (nmol/ml) before (Baseline) and 3 h after (Post) administration of 500, 2000, or 4000 mg CDP-choline ($n = 6$) or all trials combined ($n = 18$). Data are the mean ± SEM. The asterisks (*) indicate statistically significant differences between old and young: $*P < 0.05$, $**P < 0.005$, $***P < 0.0001$, by ANOVA. The dagger (‡) indicates a statistically significant difference between baseline and post-treatment values ($P < 0.005$)

Dose	500 mg		2000 mg		4000 mg		Combined	
	Baseline*	Post**	Baseline	Post*	Baseline	Post	Baseline**	Post***
Young	7.6 ± 0.63	8.0 ± 0.54	7.8 ± 0.66	8.9 ± 0.59	7.9 ± 0.66	9.2 ± 0.82	7.7 ± 0.35	8.7 ± 0.38
Old	9.8 ± 0.68	11.3 ± 0.66	9.1 ± 0.18‡	11.0 ± 0.44‡	9.1 ± 0.76	10.6 ± 0.60	9.3 ± 0.33	11.0 ± 0.32

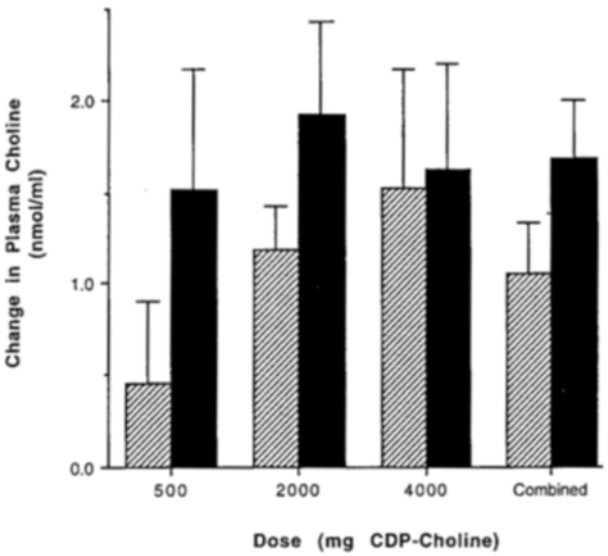

Fig. 2 Change in plasma choline 3 h after treatment with 500, 2000, or 4000 mg CDP-choline ($n = 6$ for each group) or all three doses combined ($n = 18$). Data are the mean ± SEM difference between post-treatment and pre-treatment values. There is no significant difference between old and young. ▨ Younger subjects; ■ older subjects

increases 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

Table 2 Plasma PtdCho levels (nmol/ml) before (Baseline) and 3 h after (Post) administration of 500, 2000, or 4000 mg CDP-choline ($n = 6$) or all trials combined ($n = 18$). Data are the mean ± SEM. The asterisks (*) indicate statistically significant differences between old and young: *$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$, by ANOVA

Dose	500 mg		2000 mg		4000 mg		Combined	
	Baseline*	Post**	Baseline*	Post**	Baseline	Post*	Baseline***	Post***
Young	1727 ± 74.2	1699 ± 110.8	1677 ± 82.6	1732 ± 93.3	1727 ± 111.0	1661 ± 121.2	1710 ± 49.5	1697 ± 59.6
Old	2217 ± 170.3	2221 ± 85.9	2132 ± 158.1	2314 ± 131.0	1996 ± 141.2	2196 ± 97.9	2115 ± 88.0	2244 ± 59.1

Table 3 Brain Cho/Cr before (Baseline) and 3 h after (Post) administration of 500, 2000, or 4000 mg CDP-choline ($n = 6$) or all trials combined ($n = 18$). Data are the mean ± SEM. The dagger (‡) indicates statistically significant differences between baseline and post-treatment ($P < 0.05$)

Dose	500 mg		2000 mg		4000 mg		Combined	
	Baseline	Post	Baseline	Post	Baseline	Post	Baseline	Post
Young	0.84 ± 0.025	0.96 ± 0.133	0.73 ± 0.050	0.92 ± 0.080	0.79 ± 0.048	0.89 ± 0.133	0.79 ± 0.026	0.92 ± 0.065
Old	0.86 ± 0.086	0.83 ± 0.064	0.88 ± 0.091	0.80 ± 0.071	0.90 ± 0.047‡	0.75 ± 0.034‡	0.88 ± 0.042	0.79 ± 0.033

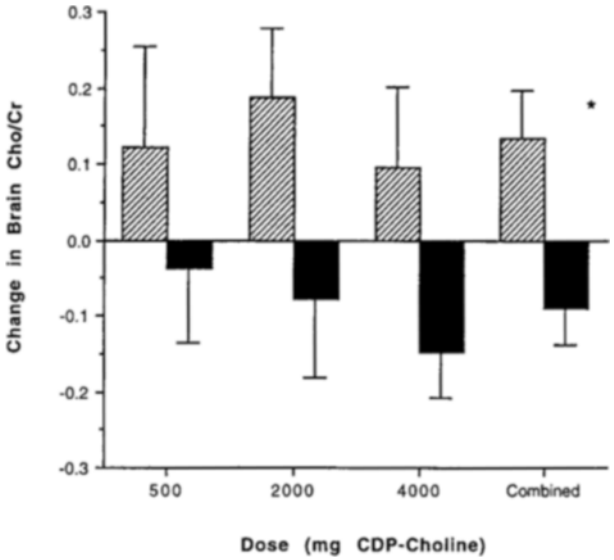

Fig. 3 Change in brain Cho/Cr 3 h after treatment with 500, 2000, or 4000 mg CDP-choline ($n = 6$ for each group) or all three doses combined ($n = 18$). Data are the mean + SEM difference between post-treatment and pre-treatment values. *Significant difference between old and young, $P = 0.017$, by repeated measures ANOVA. ▨ Younger subjects; ■ older subjects

brain Cho/Cr in female subjects was not significantly different from male subjects at any dose of CDP-choline or with all doses combined, nor were the values for either female subject ever the highest or lowest value within the older group. Within each age group, there was no difference in the change in brain Cho/Cr between doses of CDP-choline ($P = 0.68$ old; $P = 0.84$ young) and no correlation between dose and change in brain Cho/Cr ($r = 0.22$, $P = 0.37$ old; $r = 0.06$, $P = 0.83$ young).

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 distribution 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 (Savci 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 phosphocholine already present in brain into membrane PtdCho, changing the balance of MRS-visible and MRS-invisible choline containing compounds and, consequently, lowering the observed brain Cho/Cr ratio below that of the pre-treatment values. By comparison, as choline is transported readily across the blood-brain barrier in younger subjects (Cohen et al. 1994; 1995; Stoll et al. 1995), CDP-choline given to younger subjects should cause an increase both in cytidine and choline-containing compounds in brain. More MRS-visible choline-containing compounds may be formed from transported choline, and an increase over the pre-treatment values of the Cho/Cr ratio may be seen, even though cytidine is stimulating the additional synthesis of PtdCho from available choline and phosphocholine in brain.

Evidence accumulated from animal studies indicates that CDP-choline will stimulate phospholipid synthesis in brain (for review, see Weiss 1995). This study is the first to show evidence that CDP-choline can increase PtdCho synthesis in vivo 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 choline 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 1977; 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

Agut J, Lopez G-Coviella I, Ortiz JA, Wurtman RJ (1993) Oral cytidine 5′-diphosphate choline administration to rats increases brain phospholipid levels. Ann NY Acad Sci 695:318–320

Bartus RT (1990) Drugs to treat age-related neurodegenerative problems. J Am Geriatric Soc 38:680–695

Bartus RT, Dean RL, Beer B, Lippa AS (1982) The cholinergic hypothesis of geriatric memory dysfunction. Science 217: 408–410

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

Cohen BM, Zubenko GS (1985) Aging and the biophysical properties of cell membranes. Life Sci 37:1403–1409

Cohen BM, Stoll A, Renshaw P, DeMicheli E, Wurtman RJ (1994) Differences in choline uptake in brain measured in vivo by proton MRS in young and elderly adults. Soc Magn Reson Abstr 1:311

Cohen BM, Renshaw PF, Stoll AL, Wurtman RJ, Yurgelun-Todd D, Babb SM (1995) Decreased brain choline uptake in older adults: an in vivo proton magnetic resonance spectroscopy study. JAMA 274:902–907

Cornford EM, Oldendorf WH (1975) Independent blood-brain barrier transport systems for nucleic acid precursors. Biochim Biophys Acta 394:211–219

Cornford EM, Braun LD, Oldendorf WH (1978) Carrier mediated blood-brain barrier transport of choline and certain choline analogs. J Neurochem 30:299–308

De Rosa M, Galletti P, Romeo G, Nappi A, Pontoni G, Arrigoni E, Zappia V (1985) Pharmacokinetics and metabolism of double-labeled CDP choline. In: Zappia V, Kennedy EP, Nilsson BI, Galletti P (eds) Novel biochemical, pharmacological and clinical aspects of cytidinediphosphocholine. Elsevier, New York, pp 139–157

Eckernäs SA, Aquilonius SM (1977) Free choline in human plasma analysed by simple radio-enzymatic procedure: age distribution and effect of a meal. Scand J Clin Lab Invest 37:183–187

Frahm J, Bruhn H, Gyngell ML, Merboldt KD, Hänicke W, Sauter R (1989) Localized proton NMR spectroscopy in different regions of human brain in vivo. Relaxation times and concentrations of cerebral metabolites. Magn Reson Med 11:47–63

Gallai V, Mazzotta G, Firenze S, Montesi S, Del Gatto F (1991) Study of the P300 and cerebral maps in subjects with multiinfarct dementia treated with cytidine. Psychopharmacology 103:1–5

Galletti P, DeRosa M, Cotticelli MG, Morana A, Vaccaro R, Zappia V (1991) Biochemical rationale for the use of CDP-choline in traumatic brain injury: pharmacokinetics of the orally administered drug. J Neurol Sci 103:S19–S25

Grinni LS (1977) Changes in cell membranes during aging. Gerontology 23:452–464

Hartford JT, Hsu L, Samorajski T, Ordy JM (1984) Changes in blood choline and cholinergic enzymes during aging in man. In: Ordy JM, Harman D, Alfin-Slater RB (eds) Aging, vol. 26: nutrition in gerontology. Raven Press, New York, pp 227–241

Hicks P, Rolsten D, Taylor D, Samorajski T (1982) Plasma choline and blood cholinesterases in aged rats. Gerontology 28: 104–107

Jellinger K, Kienzl E, Puchinger L, Stachelberger H (1993) Changes of phospholipids in Alzheimer's disease brain. In: Corain B, Iqbal K, Nicolini M, Winblad B, Wisniewski H, Zatta P (eds) Alzheimer's disease: advances in clinical and basic research. Wiley, New York pp 315–323

Kalaria RN, Harik SI (1986) Nucleoside transporter of cerebral microvessels and choroid plexus. J Neurochem 47:1849–1856

Kang YS, Terasaki T, Ohnishi T, Tsuji A (1990) In vivo and in vitro evidence for a common carrier mediated transport of choline and basic drugs through the blood-brain barrier. J Pharmacobio-Dyn 113:353 -360

Kennedy EP, Weiss SP (1956) The function of cytidine coenzymes in the biosynthesis of phospholipids. J Biol Chem 222:193–214

Klein J, Köppen A, Löffelholz K (1991) Uptake and storage of choline by rat brain: Influence of dietary choline supplementation. J Neurochem 57:370–375

Klein J, Köppen A, Löffelholz K, Schmitthenner J (1992) Uptake and metabolism of choline by rat brain after acute choline administration. J Neurochem 58:870–876

Klein J, Gonzalez R, Köppen A, Löffelholz K (1993) Free choline and choline metabolites in rat brain and body fluids: sensitive determination and implications for choline supply to the brain. Neurochem Int 22:293–300

Löffelholz K, Klein J, Köppen A (1993) Choline, a precursor of acetylcholine and phospholipids in the brain. Prog Brain Res 98:197–200

Lopez G-Coviella I, Agut J, Wurtman RJ, Robinson, L, Ortiz JA (1988) Precursor control of phospholipid metabolism in aged mice and in neurally-derived cells in culture. Second International Serling Symposium on the Biology of Aging, Weizman Institute of Science, Rehovot, Israel, p 73

Miller BL (1991) A review of chemical issues in ^{1}H NMR spectroscopy: N-acetyl-L-aspartate, creatine and choline. NMR Biomed 4:47–52

Miller LG (1990) Dietary choline alteration: implications for γ-aminobutyric acid and other neurotransmitter receptors. Biochem Pharmacol 40:1179–1182

Millington WR, Wurtman RJ (1982) Choline administration elevates brain phosphorylcholine concentrations. J Neurochem 38: 1748–1752

Mooradian, AD (1988) Blood-brain barrier transport of choline is reduced in the aged rat. Brain Res 440:328–332

Pelech SL, Vance DE (1984) Regulation of phosphocholine biosynthesis. Biochim Biophys Acta 779:217–251

Pepeu G, Giovannelli L (1994) The central cholinergic system during aging. In: Bloom F (ed) Progess in brain research, vol. 100. Elsevier, Amsterdam, pp 67–71

Petroff OAC (1988) Biological ^{1}H NMR spectroscopy. Comp Biochem Physiol [B] 90:249–260

Petroff OAC, Ogino T, Alger JR (1988) High-resolution proton magnetic resonance spectroscopy of rabbit brain: Regional metabolite levels and postmortem changes. J Neurochem 51:163–171

Petroff OAC, Spencer DD, Alger JR, Prichard JW (1989) High-field proton magnetic resonance spectroscopy of human cerebrum obtained during surgery for epilepsy. Neurology 39:1197–1202

Plagemann PGW (1971) Nucleoside transport by Novikoff rat hepatoma cells growing in suspension culture. Biochim Biophys Acta 233:688 -701

Roth GS, Joseph JA, Mason RP (1995) Membrane alterations as causes of impaired signal transduction in Alzheimer's disease and aging. Trends Neurosci:18:203–206

Rouser G, Yamamoto A (1968) Curvilinear regression course of human brain lipid composition changes with age. Lipids 3:284–287

Savci V, Wurtman RJ (1995) Effect of cytidine on membrane phospholipid synthesis in rat striatal slices. J Neurochem 64: 378–384

Scremin OU, Jenden DJ (1993) Acetylcholine turnover and release: the influence of energy metabolism and systemic choline availability. Prog Brain Res 98:191–195

Secades JJ, Frontera G (1995) CDP-choline: Pharmacological and clinical review. Meth Find Exp Clin Pharmacol 17 [B]:1–54

Stoll AL, Renshaw PF, DeMicheli E, Wurtman R, Pillay SS, Cohen BM (1995) Choline ingestion increases the resonance of choline-containing compounds in human brain: an in vivo proton magnetic resonance study. Biol Psychiatry 37:170–174

Tucek S (1984) Problems in the organization and control of acetylcholine synthesis in brain neurons. Prog Biophys Mol Biol 44:1–46

Ulus IH, Wurtman RJ, Mauron C, Blusztajn, JK (1989) Choline increases acetylcholine release and protects against the stimulation-induced decrease in phosphatide levels within membranes of rat corpus striatum. Brain Res 484:217–227

Webb PG, Sailasuta N, Kohler SJ, Raidy T, Moats RA, Hurd RE (1994) Automated single-voxel proton MRS: technical development and multisite verification. Magn Reson Med 31: 365–373

Weiss GB (1995) Metabolism and actions of CDP-choline as an endogenous compound and administered exogenously as citicoline. Life Sci 56:637–660

Whitehead FW, Trip E, Vance DE (1981) Semliki Forest virus does not inhibit phosphatidylcholine biosynthesis in BHK-21 cells. Can J Biochem 59:38–47

Wurtman RJ (1992) Choline metabolism as a basis for the selective vulnerability of cholinergic neurons. Trends Neurosci 15:117 -122

Yashima K, Takamatsu M, Okuda K (1975) Intestinal absorption of cytidine diphosphate choline and its changes in the digestive tract. J Nutr Sci Vitaminol [Tokyo] 21:49–60

Zeisel SH (1992) Choline: an important nutrient in brain development liver function and carcinogenesis. J Am Coll Nutr 11: 473–481

Zeisel SH, daCosta KA (1990) Choline: determination using gas chromatography/mass spectrometry. J Nutr Biochem 1:55–59