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9 Choline

Choline has multiple roles as an essential nutrient. A major dietary component found in eggs and liver, its absorption in the intestine is mediated by choline transporters. The majority of choline is used to synthesize phosphatidylcholine, the predominant lipid in cell membranes. As well as being essential in the synthesis of membrane components, choline accelerates the synthesis and release of acetylcholine, an important neurotransmitter involved in memory storage and muscle control. Choline is an essential element in neurodevelopment. As a major dietary source of methyl groups, choline also participates in the biosynthesis of lipids, regulation of metabolic pathways, and detoxification in the body.

Health outcomes associated with choline involve memory, heart disease, and inflammation, which also explain the consideration of choline as a plausible intervention in traumatic brain injury (TBI). Although there are no human studies examining the effect of supplementation during pregnancy on enhanced memory of the newborn, there are animal studies showing that choline supplementation provided during hippocampal development has an effect on maintaining memory in older age. This effect appears to involve changes in gene expression via gene methylation. Changes in homocysteine due to choline supplementation are also hypothesized to reduce cardiovascular disease (CVD) risk. In the Framingham Offspring Study, combined dietary intakes of choline and betaine were associated with lower concentrations of homocysteine, a marker for inflammation. During the ATTICA study, a cross-sectional survey (1,514 men and 1,528 women with no history of CVD) of health and nutrition being carried out in the region of Attica, Greece, the association between inflammatory markers and choline intakes was measured. Participants who consumed higher levels of choline (> 310 vs. < 250 mg/day) had lower concentrations of C-reactive protein, interleukin-6, and tumor necrosis factor-alpha (Detopoulou et al., 2008). For an overview of the metabolism, functions, and health effects of choline, the reader is referred to previous reviews (IOM, 1998; Zeisel, 2006; Zeisel and da Costa, 2009; Zeisel et al., 1991).

Because of its undesirable organoleptic characteristics when administered in doses that exceed the capacity of the small intestine to absorb it, choline is not readily accepted by patients. The most common form of choline in the diet is phosphatidylcholine, an ester of choline that is not used as a substrate by gut bacteria and does not result in fishy body odor (Zeisel et al., 1983). Most studies reviewed in this chapter used an intermediary in the synthesis of phosphatidylcholine, CDP-choline. CDP-choline is composed of cytidine and choline and is hydrolyzed in the small intestine before absorption as citidine and choline. After absorption, citidine and choline are rephosphorylated and then CDP-choline is resynthesized again. CDP-choline also serves as a donor of choline in the synthesis of acetylcholine. This chapter includes evidence for the potential use of CDP-choline in TBI.

CHOLINE AND THE BRAIN

Choline has a critical role in neurotransmitter function because of its impact on acetylcholine and dopaminergic function. Studies in animals suggest that CDP-choline supplements increase dopamine receptor densities and can ameliorate memory impairment. In Parkinson's disease, for example, CDP-choline may increase the availability of dopamine. A Cochrane review of randomized trials testing the efficacy of CDP-choline in the treatment of cognitive, emotional, and behavioral deficits associated with chronic cerebral disorders in the elderly revealed no evidence of a beneficial effect on attention, but some evidence of benefit on memory function and behavior (Fioravanti and Yanagi, 2005). The brains of those with Alzheimer's disease have decreased phosphatidylcholine and phosphatidylethanolamine, and it has been suggested that CDP-choline may provide benefit by repairing cell membrane damage and enhancing acetylcholine synthesis. Both sphingomyelin and phosphatidylcholine, major

constituents of brain membranes, are synthesized from the precursor choline (Zeisel, 2005). The role of choline in regulating the synthesis of phospholipids (e.g., phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and sphingomyelin) as constituents of cell membranes is reviewed in Saver (2008). This review also includes a discussion of the evidence showing that choline promotes rapid repair of injured cell surfaces and mitochondrial membranes as well as maintenance of cell integrity and bioenergetic capacity. Increases in biomarkers representative of CDP-choline activity, such as phosphodiesters, were observed on proton magnetic resonance spectroscopy and were associated with improvements in verbal memory in humans (Babb et al., 2002; Fioravanti and Yanagi, 2005).

It is hypothesized that CDP-choline may exert neuroprotective effects in an injured brain through its ability to improve phosphatidylcholine synthesis (Adibhatla and Hatcher, 2002). In addition to its neuroprotective capability, CDP-choline potentiates neurorecovery, which has led to its evaluation as treatment for both stroke and TBI in animal models and in human clinical trials (Cohadon et al., 1982; Levin, 1991; Warach et al., 2000). The positive effects seen in models of ischemia and hypoxia may be explained by increased Bcl-2 expression, decreased apoptosis, and reduced expression of pro-caspase. Inhibiting caspase activity may decrease apoptotic activity and calcium-mediated cell death. Supporting these ideas, in vitro studies have also revealed that choline deficiency induces apoptosis in the liver by mechanisms independent of protein 53, which likely involve abnormal mitochondrial membrane phosphatidylcholine, leakage of oxygen radicals, and activation of caspases (Albright and Zeisel, 1997; Albright et al., 1996, 1998, 1999a, 199b, 2003; Chen et al., 2010). In humans, a choline-deficient diet also causes DNA damage and apoptosis (da Costa et al., 2006).

In addition, CDP-choline is hypothesized to attenuate the loss of phospholipid and increase in fatty acids after global and focal cerebral ischemia by preventing activation of phospholipase A2. CDP-choline may also act to protect against oxidative stress since it has been shown to increase total glutathione levels, glutathione reductase activity, decreased oxidized glutathione, and glutathione oxidation ratio (Adibhatla and Hatcher, 2005).

In rat models, the availability of choline to the fetus influences neurogenesis in the fetal brain (Craciunescu et al., 2003), and choline status in early life influences neurogenesis rates in the adult hippocampus (Glenn et al., 2007), an area of the brain that is often dysfunctional in TBI. Additionally suggesting choline mechanisms of action relevant to TBI are the fact that in rodents, choline deficiency is associated with lipid peroxidation in liver (Ghoshal et al., 1984, 1990) and that deletion of a choline metabolism gene results in mitochondrial dysfunction in the liver, sperm, testis, heart, and kidney (Johnson et al., 2010). A list of human studies (years 1990 and beyond) evaluating the effectiveness of CDP-choline in providing resilience or treating TBI or related diseases or conditions (i.e., subarachnoid hemorrhage, intracranial aneurysm, stroke, anoxic or hypoxic ischemia, epilepsy) in the acute phase in humans is presented in Table 9-1; this also includes supporting evidence from animal models of TBI. The table includes the occurrence or absence of adverse effects in humans.

USES AND SAFETY

In 1998, the Institute of Medicine (IOM) recognized choline as an essential nutrient (IOM, 1998; Zeisel and da Costa, 2009) and set the Adequate Intake (AI) for choline at 550 mg/day and 425 mg/day for men and women 19 years of age and older, respectively. These levels were set based on the dietary intakes of the U.S. population, and on the development of liver damage seen with lower intake. The Tolerable Upper Intake Level (UL) for choline is 3.5 g/day for adults 19 years of age or older, based on fishy body odor and hypotension (IOM, 1998).

Choline is found in a variety of foods including eggs and liver. Deficiency has been clearly linked to atherosclerosis, neurodevelopmental diseases, and liver disease (Penry and Manore, 2008). The human body is unable to synthesize sufficient choline via direct methylation of phosphatidylethanolamine to phosphatidylcholine, so choline must also be acquired via the diet. Analysis of choline intake has suggested a high level of deficiency in the U.S. population (Fischer et al., 2005; Jensen et al., 2007). Choline deficiency has been linked to a variety of secondary disease processes, such as liver disease; cardiac, neurodegenerative and neurodevelopmental problems; and breast cancer (Li

and Vance, 2008; Zeisel, 2006). In addition, it is estimated that up to 50 percent of the population carries genetic variations that require increased choline intake (Zeisel and da Costa, 2009).

Direct choline therapy, when administered in doses higher than the intestine can absorb, often leads to malodor that is unacceptable to participants. The use of forms of choline that are efficiently absorbed and avoid this problem is desirable. All the studies reported by the committee have used CDP-choline, an endogenous compound and intermediary of the synthesis of phosphatidylcholine. CDP-choline was originally identified as the key intermediary in the biosynthesis of phosphatidylcholine by Kennedy in 1956 (2003), and is now also sold as a dietary supplement. However, there is no evidence that CDP-choline is the most effective form, and other forms of choline could be tested in future TBI studies.

CDP-choline has been used in the treatment of cerebrovascular disorders for many years, under a variety of protocols and to ameliorate various conditions. In several European countries, for example, CDP-choline is frequently prescribed for cognitive impairment and in the treatment of Parkinson's disease.

CDP-choline is generally considered safe; the side effect most noted in clinical trials has been mild diarrhea, with leg edema, back pain with headache, tinnitus, insomnia, vision problems, and dizziness reported much less frequently (Adibhatla and Hatcher, 2002; Clark et al., 1997; Levin, 1991). There were no adverse events reported even with doses as high as 4,000 mg/day (Calatayud Maldonado et al., 1991). It is notable that in a study by Clark and colleagues (2001), a dose of 2,000 mg/day by enteral administration did not induce severe adverse events at a rate any higher than placebo in the 899 patients.

EVIDENCE INDICATING EFFECT ON RESILIENCE

The committee found no clinical or animal trials that have tested the potential benefits of choline or CDP-choline in TBI or in other diseases or conditions included in the reviews of the literature (subarachnoid hemorrhage, intracranial aneurysm, stroke, anoxic or hypoxic ischemia, epilepsy).

EVIDENCE INDICATING EFFECT ON TREATMENT

Human Studies

In human studies, patients who were administered CDP-choline early in the postischemia recovery process demonstrated improved levels of consciousness (Tazaki et al., 1988) as well as improvements in the modified Rankin scale (a measure of function after stroke) (Clark et al., 2001). Consistent with this observation, magnetic resonance imaging data show a decrease in lesion volume with CDP-choline compared to placebo in a preliminary trial (Warach et al., 2000). A meta-analysis was conducted of four randomized clinical trials of CDP-choline in stroke in the United States (Davalos et al., 2002). Although the conclusion from pooling the data in the meta-analysis was positive and the authors concluded that oral CDP-choline increases the probability of recovery, the results of the individual studies are ambiguous. CDP-choline improved functional outcome and reduced neurological deficit in one of those studies (Clark et al., 1997); however, two subsequent studies failed to demonstrate improvement, although a post hoc analysis showed improvements in moderate to severe stroke cases (Clark et al., 1999, 2001). One of the studies (Clark et al., 2001) showed a beneficial effect of CDP-choline as measured by the Rankin scale, a secondary outcome metric in these trials. A separate meta-analysis of acute and subacute stroke, published in abstract form only, suggested a positive treatment effect of CDP-choline precursors on rates of death and disability (Saver et al., 2002).

In early randomized clinical trials of CDP-choline in TBI, it was associated with faster recovery from focal motor deficits in patients with severe TBI (Cohadon et al., 1982); improved recall design (a measure of memory) (Levin, 1991); a reduction of postconcussion symptoms following mild TBI (Levin, 1991); and reduced inpatient hospital stay and requirement for outpatient follow-up (Calatayud Maldonado et al., 1991). CDP-choline has also been shown to

enhance cerebral blood flow. Among patients with TBI and very severe memory deficits, hypoperfusion of the inferior left temporal lobe normalized after administration of CDP-choline (Leon-Carrion et al., 2000).

Clinical trials of CDP-choline in TBI have demonstrated efficacy in secondary outcome measures but not in primary measures. These ambiguous results of some of the human trials in the United States may be due to a combination of causes. Many of the trials used doses substantially lower than may be optimal for highest efficacy (Agut et al., 1983; Clark et al., 1997). Also, this failure may have been due to substantial weaknesses in study designs, such as insufficient sample size (Calatayud Maldonado et al., 1991; Cohadon et al., 1982; Tazaki et al., 1988) or lack of sensitivity of the chosen outcomes measure (Glasgow Outcome Scale) (Clark et al., 2001). For example, Clark's study of patients with stroke did not show a significant difference in the primary outcome measure (an improvement of total score by > 7 in the National Institutes of Health Stroke Scale), but post hoc analysis using a standard of "excellent recovery" showed a possible treatment effect. In this study, the primary outcome measure may have been too stringent (Clark et al., 2001). Differences in outcomes also may have been due to the route of administration of CDP-choline. Although bioavailability data suggest that enteral and intravenous routes are similar, brain uptake of CDP-choline may vary depending on the route of administration (Adibhatla and Hatcher, 2002; Grotta, 2002; Secades and Frontera, 1995). Theoretically, it is possible that intravenous administration may yield higher brain delivery (Agut et al., 1983; Secades and Frontera, 1995).

Animal Studies

In animal models, CDP-choline has been demonstrated to exert acute neuroprotection, as well as positive effects in chronic brain injury and stroke and in epilepsy.

A major mechanism of secondary injury in TBI is the formation of reactive oxygen species and lipid peroxidases, which cause significant tissue damage. Animal models of TBI support a key role for oxidative stress (Ikeda and Long, 1990; Kontos et al., 1992). The exogenous administration of CDP-choline or its precursors significantly increased levels of glutathione (Adibhatla et al., 2001; Barrachina et al., 2003; De la Cruz et al., 2000), a powerful endogenous antioxidant. CDP-choline also attenuates release of arachidonic acid, cardiolipin, and sphingomyelin (Adibhatla and Hatcher, 2002). Studies in animal models of ischemia and hypoxia also found that CDP-choline treatment improves concentration of free fatty acids, decreases neurological deficits, and improves behavioral performance on learning and memory (Rao et al., 2001). Increased expression of B-cell lymphoma 2, a regulator of apoptosis; decreased apoptosis; and reduced expression of both pro-caspase (Krupinski et al., 2002) and cleaved caspase-3 (Mir et al., 2003) also may explain the functional find ings. Inhibiting caspase activity may decrease apoptotic activity and calcium-mediated cell death.

CDP-choline was found to be neuroprotective in an animal model of uninterrupted occlusion of the basilar artery after subarachnoid hemorrhage (Alkan et al., 2001). CDP-choline was associated with greater arterial pressure, smaller infarct volumes, and lower mortality than controls. These results also suggest that CDP-choline provides significant neuroprotection during cerebral ischemia.

Dietary choline may promote functional recovery from status epilepticus (Holmes et al., 2002; Wong-Goodrich et al., 2010). Following the status epilepticus, rats given a choline-supplemented diet for four weeks performed better on the Morris water maze test than rats receiving a control diet (Holmes et al., 2002).

Animal studies (Baskaya et al., 2000; Dempsey and Raghavendra Rao, 2003; Dixon et al., 1997) demonstrated the neuroprotective effect of CDP-choline in TBI. The studies showed that CDP-choline had a significant preventive effect on TBI-induced neuronal loss in the hippocampus, decreased cortical contusion volume, and improved neurological recovery. Additionally, there was a dose-dependent attenuation of chronic deficits in motor and spatial performance following CDP-choline administration. Extracellular levels of acetylcholine, a key mediator of memory processes, were increased (Dixon et al., 1997), suggesting that CDP-choline enhances cholinergic transmission and may ameliorate chronic functional deficits. A second mechanism that may explain why CDP-choline improves

function in chronically injured animals focuses on observed decreases in dopamine following injury (Yan et al., 2001). In such models, CDP-choline increased dopamine levels (Secades and Frontera, 1995; Yan et al., 2001), which enhanced neurorecovery (Kline et al., 2004).

CONCLUSIONS AND RECOMMENDATIONS

Since 2000, several neuroprotective trials for TBI have failed to show efficacy in any of the interventions tested. One reason may be that many of these agents have targeted one portion of the cascade of injury that occurs after TBI. Such agents have a time-limited opportunity to prevent the secondary brain injury and are rarely involved in the restorative process. An ideal agent would provide both neuroprotection and a means to facilitate the recovery process.

Although clinical trials in stroke and trauma have suggested efficacy in secondary outcome measures related to functional outcome and cognition, design weaknesses in these studies may have affected findings in the primary outcome. Design limitations include insufficient sample size (Tazaki et al., 1988), low dosage (Clark et al., 1997, 1999), variations derived from intravenous versus enteral delivery (Calatayud Maldonado et al., 1991; Clark et al., 2001), and in some cases inadequate outcome measures (Clark et al., 2001).

Preliminary animal data suggest that CDP-choline works via numerous mechanisms to limit the acute secondary injury cascade after ischemic and traumatic injury. In the more chronic setting, CDP-choline appears responsible for an upregulation in acetylcholine synthesis. The diversity of CDP-choline's mechanisms of action suggests that it may offer neuroprotection and neurofacilitation to patients with TBI through multiple avenues, thereby increasing the possibility of that treatment improving outcome. The optimal clinical dose and duration of treatment of CDP-choline for injured patients is yet unclear.

There is one ongoing human trial on the effect of CDP-choline (Citicoline Brain Injury Treatment [COBRIT] trial) on cognition and functional measures on severe, moderate, and complicated mild TBI being led by a member of the committee (Zafonte et al., 2009). The committee recognizes the significance of this trial in that the findings will reveal more insights about the potential for this nutrient in the treatment of TBI. It was the consensus of the committee to emphasize the importance of monitoring the results of this trial before conducting more human studies. If ongoing trials with CDP-choline and TBI patients show positive results, further studies are warranted to confirm the optimal duration of treatment and clinical dose of choline for injured patients. Likewise, if those studies reveal that choline is a promising intervention, the effect of choline supplementation prior to injury to improve resilience could be explored by conducting animal studies. The impact on neurologic outcome of the choline deficiency observed in the population needs to be explored. Although there are no data regarding supplementation to enhance resilience, choline's critical role in the maintenance of health suggests that individuals should be cautioned to avoid deficiency. Based on findings from animal studies, it would be prudent to consider potential gender differences in the metabolism of choline when designing studies (Fischer et al., 2007; Resseguie et al., 2007, 2011).

RECOMMENDATION 9-1. DoD should monitor the results of the COBRIT trial, a human experimental trial examining the effect of CDP-choline and genomic factors on cognition and functional measures in severe, moderate, and complicated mild TBI. If the results of that trial are positive, then DoD should conduct animal studies to define the optimal clinical dose and duration of treatment for choline (CDP-choline) following TBI, as well as to explore choline's potential to promote resilience to TBI when used as a preinjury supplement.

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Tables

TABLE 9-1 Relevant Data Identified for Citicoline/CDP-Choline

Reference	Type of Injury/Insult	Type of Study and Subjects	Treatment	Findings/Results
Tier 1: Clinical trials				
Zafonte et al., 2009	Mild, moderate, and severe TBI	Randomized, double-blind, placebo-controlled, multi-center trial	Postinjury, 90 days treatment of citicoline (1,000 mg twice a day), administered enterally or orally	Trial in progress
		n[a]=1,292		
Stroke	Saver, 2008	Meta-analysis; 10 trials	CDP-choline	Mortality and disability rates are lower in CDP-choline-treated patients than in placebo patients (OR[b]=0.64, 95% CI[c]: 0.54–.077, p < 0.00001; p for heterogeneity=0.01, χ^{2d}=21.40).
		n=2,279		Due to large amount of scatter in smaller studies, another analysis of the 4 largest studies (n > 100) was conducted; treatment effect on mortality and disability was still significant (OR=0.70, 95% CI: 0.58–0.85; p=0.0003).
				In patients with NIHSS (National Institutes of Health Stroke Scale) ≥ 8, overall recovery occurred more often in CDP-choline-treated patients (OR=1.30, 95% CI: 1.1–1.6; p < 0.004). More CDP-choline patients (25.2%) achieved NIHSS of 0–1, Barthel Index of ≥ 95, and modified Rankin Score of 0–1 than placebo patients (20.2%).
				There were no adverse effects observed due to the treatments.
Davalos et al., 2002	Moderate to severe stroke	Pooled data analysis; randomized, placebo-controlled, double-blind clinical trials	Postinjury; oral CDP-choline (500 mg, 1,000 mg, and 2,000 mg) vs. placebo; treatment within 24 hours after injury	Global recovery after 3 months was seen in 25.2% of CDP-choline-treated patients and 20.2% of placebo-treated patients (OR=1.33; 95% CI: 1.10–1.62; p=0.0034). Greatest improvement was seen in 2000 mg group, 27.9% (OR=1.38, 95% CI: 1.10–1.72, p=0.0043). Compared to placebo-group, CDP-choline-treated group saw an increase of 29% on Barthel Index
		n=1,372 (583=placebo; 789=treatment)		score (95% CI: 3–62), 42% in modified Rankin Score (95% CI: 8–88), and 28% on NIHSS (95% CI: –1 to 65).
				There is no significant mortality rate and overall

Reference	Type of Injury/Insult	Type of Study and Subjects	Treatment	Findings/Results
				There is no significant mortality rate and overall frequency of adverse events between treated and placebo groups. Significantly higher events were found in the treatment group for anxiety and leg edema (p=0.036 and 0.032, respectively).
Clark et al., 2001	Acute ischemic stroke, NIHSS ≥ 8	118-center, randomized, double-blind, efficacy trial	Postinjury; CDP-choline (2,000 mg per day); 6-week treatment, 6-week follow-up	At week 12, about the same proportion of patients in placebo (51%) and treatment (52%) groups showed a 7-point improvement on their NIHSS scores.
		n=899		Although the treatment group did significantly better (27% vs. 21%; p=0.04) than placebo group on the Barthel Index at week 6, it lost that advantage at week 12.
				There was no significant difference in mortality rate or other serious adverse events (e.g., cardiovascular events, central nervous system events) between the treatment and placebo groups.
Leon-Carrion et al., 2000	TBI	**Exp 1:** Non-randomized trial	**Exp 1:** 1 g of CDP-choline	**Exp1:** Patients showed a hypoperfusion of the inferior left temporal lobe at resting state, but hypoperfusion disappeared after taking CDP-choline.
		n=7 patients with severe memory disorders who were discharged > 6 months prior to study	**Exp 2:** 1 g/day of CDP-choline or placebo administered with patients' neuropsychological treatment	Cerebral blood flow increased in the left temporal areas and decreased in right frontal lobe.
		Exp 2: Randomized trial		**Exp2:** While the CPD-choline group improved in all 5 measures of the neuropsychological treatment, only improvements in verbal fluency and Luria Memory Words were significant ($p < 0.05$).
		n=10 patients with severe memory deficits (including the 7 patients from Exp 1)		There were no side effects reported for patients in Exp 1. There were no observed side effects in Exp 2.
Warach et	Acute	Randomized,	Post-injury, CDP-	From baseline to week 12, the distribution of

Reference	Type of Injury/Insult	Type of Study and Subjects	Treatment	Findings/Results
al., 2000	stroke and lesions of 1–120 cc in cerebral gray matter	double-blind, placebo-controlled trial n=100, onset 24 hrs or less	choline (500 mg/day) or placebo administered orally for 6 weeks, follow-up for 12 weeks	changes in ischemic region volume was not significantly different between placebo group and CDP-choline-treat group. However, CDP-choline group showed significantly greater reduction ($p < 0.01$) in analysis of week 1 to week 12 change. Significant ($p \leq 0.0001$) covariates of change in lesion volume are: size of baseline perfusion abnormality, baseline NIHSS score, and presence of arterial lesion seen on MRA. Patients' improvement of NIHSS ≥ 7 points showed greater lesion volume reduction ($p \leq 0.001$).
				The difference in mortality rate between the treatment and placebo group was not significant. Edema of the extremities and back pain were significantly higher in the CDP-choline group than in the placebo group ($p \leq 0.05$ for both).
Clark et al., 1999	Acute ischemic stroke, NIHSS ≥ 5	Randomized, double-blind, efficacy trial at 31 centers n=394 (127=placebo, 267=CDP-choline)	Postinjury, oral CDP-choline (500 mg/day); 6 weeks of treatment, 6 weeks of follow-up	Post hoc analyses found that among patients with baseline NIHSS ≥ 8, CDP-choline-treated patients were overall more likely to have a full recovery (OR=1.9, p=0.04). No treatment effect was seen in patients with baseline NIHSS < 8.
				CDP-choline-treated patients were significantly (p=0.01) more likely to achieve a 7-point improvement in NIHSS score than placebo-treated patients.
				There was no significant difference in mortality rate or other serious adverse events between the treatment and placebo groups.
Clark et al., 1997	Acute ischemic stroke, NIHSS ≥ 5	Randomized, double-blind, placebo-controlled trial at 21 centers n=259	6 weeks of CDP-choline (50 mg, 1,000 mg, or 2,000 mg daily) or placebo	Primary analysis using NIHSS as a covariate showed that, overall, CDP-choline had treatment effect compared to placebo ($p \leq 0.05$).
				The 500-mg group (OR=2.0) and the 20,00-mg group (OR=2.1) achieved a significantly ($p < 0.05$) higher Barthel Index score at week 12. Overall, CDP-choline treatment at week 12 was associated with full recovery, as defined by Barthel Index of ≥ 95 (p=0.011); specifically, the 500-mg group had a significant (p=0.03) improvement compared to placebo group.
				The 500-mg group also significantly (p=0.03) improved on Rankin Scale score. Treatment effect on cognitive function (MMSE ≥ 25) at 12 weeks was seen in 500-mg group (OR=2.6, p=0.02) and the 2,000-mg group (OR=2.4, p=0.03).
				[illegible]

Reference	Type of Injury/Insult	Type of Study and Subjects	Treatment	Findings/Results
				There was no significant difference in mortality rate or other serious adverse events between the treatment and placebo groups. Adverse events that were significantly higher in the treatment groups than in the placebo group were dizziness and accidental injury ($p \leq 0.05$).
Levin, 1991	Mild to moderate closed head injury	Randomized, double-blind, placebo-controlled trial	Postinjury, oral CDP-choline (1 g) or placebo	Patients treated with CDP-choline had greater improvement (100%) on tests recalling designs than placebo-treated patients (29%, $p < 0.02$).
		n=14 men		While placebo-treated patients have higher absolute score on tests to create unique designs than CDP-choline treated patients ($p < 0.05$) during the 1-month follow-up, the change in scores from baseline was not significantly different between the two groups.
				Although CDP-choline was well tolerated, there were more complaints about gastrointestinal distress from patients in the treatment group.
Maldonado et al., 1991	Severe and moderate closed TBI, GCS (Glasgow Coma Score) between 5 and 10	Randomized, single-blind trial	Conventional treatment vs. CDP-choline added to conventional treatment; follow-up after 3 months	Patients treated with CDP-choline had shorter hospital stays than control patients ($p < 0.05$). CDP-choline group showed overall improvement in all initial symptoms, but only the improvement in character was significant ($p < 0.05$).
		n=216		CDP-choline patients also showed significantly better results on GOS (Glasgow Outcome Score) ($p=0.05$). There was no significant difference between groups in terms of mortality.
				There were no adverse effects reported.
Tier 2: Observational studies				
None found				
Tier 3: Animal studies				
Dempsey and Raghavendra Rao, 2003	Moderate-grade TBI, controlled cortical impact (CCI)	Adult, male Sprague-Dawley rats	Postinjury, intraperitoneal injections of CDP-choline (100, 200, or 400 mg/ kg body weight) or	Compared to sham-injured rats, injured rats treated with saline and 100 mg/kg of CDP-choline had greater neuron loss in the CA2 and CA3 regions of the hippocampus ($p < 0.05$ for both). However, treatment with 200 mg/kg and 400 mg/kg of CDP-choline reduce the loss in the same regions ($p < 0.05$ vs. injured, saline-treated

Reference	Type of Injury/Insult	Type of Study and Subjects	Treatment	Findings/Results
			saline < 3 minutes postinjury and 6 hours postinjury	saline regions ($p < 0.05$ vs. injured, saline-treated rats).
				Treatment with 200 mg/kg and 400 mg/kg of CDP-choline also reduced the volume of cortical contusion by 21 mm^3 ($p < 0.05$).
				Rats treated with 200 or 400 mg/kg of CDP-choline significantly recovered their neurological function by day 7 to 88% of their preinjury level ($p < 0.05$).
Baskaya et al., 2000	TBI, CCI	Adult, male Sprague-Dawley rats	Postinjury, intraperitoneal injections of saline or CDP-choline (50, 100, or 400 mg/ kg body weight) administered 5 minutes and 4–6 hours after injury	100 mg/kg CDP-choline significantly reduced edema in the cortex ($p < 0.05$ vs. saline treatment), while 400 mg/kg CDP-choline significantly reduced edema in both the cortex and the ipsilateral hippocampus ($p < 0.05$ vs. saline treatment).
				Doses of 100 and 400 mg/kg body weight CDP-choline significantly ($p < 0.05$) reduced blood-brain barrier breakdown in both the injured cortex and ipsilateral hippocampus.
Dixon et al., 1997	TBI, lateral CCI	Adult, male Sprague-Dawley rats	Postinjury, daily intraperitoneal injection of CDP-choline (100 mg/ kg) for	Compared to injured, saline-treated rats, CDP-choline-treated rats had greater latency on beam balancing task ($p < 0.01$) and shorter latency beam walking task ($p < 0.05$) at day 1. The difference between two groups in both tasks was minimized by day 4.
			18 days, beginning 1 day postinjury	CDP-choline treated rats also had shorter latency in completing the Morris water maze than saline-treated rats ($p < 0.005$).
				Acetylcholine outflow was significantly increased in the dorsal hippocampus ($p < 0.014$) and neocortex ($p < 0.036$) after treatment with CDP-choline.

a n: sample size.

b OR: odds ratio.

c CI: confidence interval.

d $\chi 2$: chi-square.

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