CDP-choline for acute and subacute ischemic stroke. An updated and cumulative meta-analysis (Protocol)

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BACKGROUND
Cell membranes are composed of phospholipid bilayers, including phosphatidylcholine, the most important membrane phospholipid in mammals. CDP-choline or citicoline is an exogenous agent that is converted to choline and cytidine in the body and promote the maintenance, repair, and de novo formation of cell membrane phospholipids, as well as the neurotransmitters acetylcholine and dopamine. Also CDP-choline has shown reparative effects. CDP-choline has been studied as treatment for acute ischemic stroke for over four decades and is widely available worldwide, being registered and marketed in around 70 countries.

Description of the condition
Stroke is the second leading cause of death and a leading cause of disability worldwide. Damage to cell membrane integrity and the alteration of the lipidic metabolism are important events leading to cell death in ischemic stroke. A treatment strategy aimed at protecting and regenerating cell membranes, and normalizing the lipidic metabolism may be beneficial following stroke.

Description of the intervention
Citicoline or CDP-choline (cytidine 5’-diphosphocholine, CDP-choline) is a naturally occurring, endogenous compound that is the rate-limiting intermediate in the Kennedy cycle for the biosynthesis of phosphatidylcholine, the major neuronal membrane lipid (Kennedy 1956; Vance 1990; Agut 1993). The synthesis of 80% of central nervous system phospholipids can be controlled by altering the concentration of citicoline (Dorman 1982). When administered exogenously, citicoline is rapidly hydrolyzed in the intestine and the circulation to form choline and cytidine. While only small amounts of
circulating choline and cytidine cross the blood-brain barrier, the fraction that reaches the central nervous system is efficiently employed to generate phospholipids (Galletti 1991; Adibhatla 2006). A substantial body of literature in vivo stroke models supports a therapeutic effect of citicoline. Benefits have been reported in over 12 different ischemia/hypoxia models in more than five species, although many older studies examined only indirect indices of ischemic insult (Weiss 1995; Aronowski 1996; Schabitz 1996; Shuaib 2000; Hurtado 2008).

**How the intervention might work**
CDP-choline may benefit acute and subacute ischemic stroke patients through a variety of mechanisms, including:

1. stabilizing damaged cell membranes (Adibhatla 2005; Secades 2010);
2. diminishing the generation of damaging free fatty acids and free radicals (Rao 2000; Adibhatla 2003; Adibhatla 2007; Adibhatla 2010);
3. enhancing neurorepair and neuroplasticity by promoting axonal sprouting and forming new synaptic connections (Wurtman 2006; Hurtado 2007; Secades 2011);
4. enhancing neurorepair and neuroplasticity by promoting neurogenesis and receptor up-regulation (Diederich 2012)
5. enhancing neurorepair and neuroplasticity by promoting angiogenesis (Krupinski 2012)
6. relieving symptoms due to diminished cholinergic and dopaminergic neurotransmission by increasing the acetylcholine and dopamine neurotransmitter levels (D'Orlando 1995; Spiers 1996; Parnetti 2001; Secades 2011; Secades 2012).

**Why it is important to do this review**
Several studies of citicoline in acute and subacute stroke have been conducted. Most of these studies performed to date were underpowered to detect a modest, but clinically worthwhile, benefit. A pooled analysis of a subset of individual patient data extracted from four oral citicoline trials suggested a modest benefit in the citicoline treated group (Dávalos 2002). After the neutral results of the most recent clinical trial performed (Dávalos 2012) and given that citicoline is widely used in clinical practice in ischemic stroke patients in many areas of the world, a formal updated meta-analysis of the entirety of clinical trial data is an important undertaking. Also we will perform a cumulative meta-analysis to show how the effect of the treatment has changed across the time.

**OBJECTIVES**
To assess the benefits and hazards of therapy with CDP-choline in acute and subacute ischemic stroke through a systematic review of randomized controlled trials.

METHODS
Criteria for considering studies for this review

Types of studies
We will only include trials that are double-blind, randomized and controlled with placebo.

Types of participants
We will include all trials that included patients of any age or sex with index events of ischemic stroke and/or presumed ischemic stroke (in studies without neuroimaging), with randomization occurring within 14 days after stroke onset. As the treatment may have effects at different stages in the evolution of ischemic brain injury and repair, we propose a broad treatment time window of 14 days.

Types of interventions
We will include all trials in which the active study agent was CDP-choline. We will exclude trials that CDP-choline treatment is compared with any other active treatment.

Types of outcome measures
The primary efficacy measure will be independency at the end of scheduled clinical trial follow up. If available, we will use the modified Rankin Scale (mRS) for this measure (mRS 0-2). In studies lacking the Rankin measure, we will employ the most comprehensive measure of disability or handicap available from the trial.

Search methods for identification of studies

Electronic searches
We will search the Cochrane Stroke Group Trials Register. In addition, we will search the following electronic bibliographic databases and trial registers:
- Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library, latest issue);
- MEDLINE (1966 to present)
- EMBASE (1974 to present);
- Internet Stroke Center stroke trials registry;
- National Institute of Health: ClinicalTrials.gov
**MEDLINE search strategy**

We will use the following search strategy for MEDLINE and modify it for the other databases.

MEDLINE (Ovid)
1. cerebrovascular disorders/ or basal ganglia cerebrovascular disease/ or exp brain ischemia/ or carotid artery diseases/ or carotid artery thrombosis/ or intracranial arterial diseases/ or cerebral arterial diseases/ or exp “intracranial embolism and thrombosis”/ or stroke/ or exp brain infarction/or cerebral infarction/
2. ((ischaemic or ischemic) adj5 (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva)).tw.
3. ((brain or cerebr$ or cerebell$ or vertebrobasil$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw.
4. 1 or 2 or 3
5. cdp-choline/ or cytidine diphosphate choline/ or citicoline
6. (choline or (cytidine adj5 diphosphocholine) or cytidine diphosphate choline or CDP-choline or citicoline or citicholine or cyticholine or cidifos).tw.
7. 5 or 6
8. 4 and 7
9. limit 8 to humans

**Searching other resources**

In an effort to identify further published, unpublished and ongoing trials we will:
1. search the reference lists of all relevant articles found;
2. use Ferrer Group bibliographic database, to obtain any additional information not available in published trial reports;
3. contact authors and researchers active in the field.
4. do an exhaustive search in internet to detect studies published in not indexed journals.
We will not apply any language restriction, and we will have relevant articles published in languages other than English translated.

**Data collection and analysis**

**Selection of studies**

Three review authors (JJS, JD and NO) will review the abstracts of articles retrieved in the search. We will obtain full-length papers for any abstract we feel possibly meets the inclusion criteria. Four review authors (JJS, JC, EDT, EMV) will review all the retrieved papers to identify those trials meeting the inclusion criteria for the review.
Data extraction and management
Three review authors (JJS, JD and NO) will independently abstract the efficacy data from eligible trials. They will resolve discrepancies by discussion with the other authors (JC, EDT and EMV) and by referencing the original report.

Assessment of risk of bias in included studies
We will assess the methodological quality of selected studies using The Cochrane Collaboration’s tool for assessing risk of bias (Higgins 2008). We will score each of the following points as ‘yes’, ‘no’, or ‘unclear’ (where ‘yes’ indicates that the study is less open to bias):

1. Method of randomization (selection bias).
2. Concealment of allocation (indication bias).
3. Blinding of investigators and patients (performance bias).
5. Adequate follow up (attrition bias).
6. Other possible bias.

Based on these criteria, we will divide studies into the following three categories:
• A - all quality criteria met: low risk of bias;
• B - one or more of the quality criteria only partly met: moderate risk of bias;
• C - one or more criteria not met: high risk of bias.

To avoid selection bias, we will only reject studies categorized as C, including non-randomized studies. Other studies not included will be those made for other indications, such as hemorrhagic stroke, and those not compared with placebo.

Measures of treatment effect
We will employ dichotomous statistical meta-analytic methods. We will use the Peto odds ratio (OR) to test for proportional treatment effects.

Assessment of heterogeneity
As it is presumed heterogeneity among studies performed in 4 decades, the main analysis will use the random-effects model to determine if the effects of CDP-choline are statistically significantly different from control. Also the fixed-effects model will be used to compare the estimates. If the estimates are similar, then any small-study effects have little effect on the intervention effect estimate (Higgins 2008).
**Data synthesis**
The formal meta-analysis will be performed using the \texttt{rmeta} package (Lumley T (2009). \texttt{rmeta: Meta-Analysis.} R package version 2.16, URL \url{http://CRAN.R-project.org/package=rmeta}. of the \texttt{R} software (R Development Core Team (2005). \texttt{R: A language and environment for statistical computing}. \texttt{R} Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL \url{http://www.R-project.org}).

For the cumulative meta-analysis we will use the specific software MetaAnalyst Beta 3.13.

**Subgroup analysis**
No subgroup analyses are planned.

**Sensitivity analysis**
If evidence of small-study effects is observed, we will perform sensitivity analyses. When there is evidence of between-study heterogeneity ($I^2 > 50\%$), we will compare the fixed-effect and random-effects estimates of the intervention effect.

**REFERENCES**

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Adibhatla 2010

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Aronowski 1996

D’Orlando 1995

Dávalos 2002

Dávalos 2012

Diederich 2012

Dorman 1982

Galletti 1991

Higgins 2008

Hurtado 2007

Hurtado 2008

Kennedy 1956

Krupinski 2012

Parnetti 2001

Rao 2000
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CONTRIBUTIONS OF AUTHORS

Draft the protocol JJS, JD and NO
Approve the protocol JC, EDT and EMV
Develop a search strategy  JJS
Search for trials  JJS, JC, EDT and EMV
Obtain copies of trials  JJS
Select which trials to include  JJS, JC, EDT and EMV
Initial extract data from trials  JJS, JD and NO
Confirm data extracted from trials  JC, EDT and EMV
Carry out formal m-a  NO
Carry out cumulative m-a  JJS
Interpret the analysis  JJS, JD, JC, EDT and EMV
Draft the final review  JJS and NO
Approve the final review  JD, JC, EDT and EMV