Neuroprotection and Recovery
Recent Data at the Bench on Citicoline

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Background and Purpose—In this work, we review recent data on the actions of citicoline, citicoline is a drug with demonstrated neuroprotective properties in both animals and humans.

Summary of Review—For neuroprotection, mechanisms involved are the improvement of cellular functions aimed to control excitotoxicity and to maintain cellular adenosine 5'-triphosphate levels by preserving membrane function and integrity at different levels. Importantly, these actions are theoretically achieved without interfering with possible underlying mechanisms for neurorepair. Furthermore, citicoline stimulates neuronal plasticity and improves sensorimotor recovery in the chronic phase of experimental stroke.

Conclusions—Although the mechanisms of some of these actions remain to be elucidated, so far citicoline appears as a drug with the ability to promote “safe” neuroprotection capable of enhancing endogenous protective pathways at the same time as preparing the scenario for plasticity. (Stroke. 2011;42[suppl 1]:S33-S35.)

Key Words: functional recovery • glutamate • neuroprotection • neuroregeneration

In acute stroke management, current evidence demonstrates that the most important declines in stroke incidence and mortality in developed countries have evolved from primary and secondary prevention measures aimed to better control of risk factors, including either pharmacological treatments or other procedures that decrease blood pressure, prevent consequences of atrial fibrillation, and reduce hyperglycemia and hyperlipidemia, among others.

Although less successful, there have also been interventions with proven benefit for acute stroke treatment. One of the most significant advances has been the management of patients in stroke units, which has been shown to reduce mortality and to improve functional outcome by approximately 20%.1 In addition, recanalization of the occluded blood vessel with thrombolytics, mainly recombinant tissue plasminogen activator and, more recently, by mechanical clot removal or disruption, appears to be 1 of the most effective treatments for acute ischemic stroke.

However, there have been major disappointments in the area of pharmacological neuroprotection, where many clinical trials have so far failed. Some of these failures might be due to deficiencies in trial design rather than absence of efficacy of the agents tested. In this sense, many current efforts are now devoted to develop methods for better patient selection and for analysis of outcomes, a way in which recent trials have begun to provide some hope. Still, acute stroke treatment guidelines until now do not encourage treatment with any neuroprotectant.

Lastly, there is a field for which there are hopes that some interventions could be useful for patients with stroke, which is the phase of recovery. Indeed, in this setting, strategies including rehabilitation programs or pharmacological/cell therapies may serve to promote repair in late phases and to decrease stroke-associated disability.

A way to embark on the search for new drugs for the chronic phase of stroke is by boosting endogenous plasticity mechanisms. Interestingly, many of these endogenous active recovery processes initiate very early, in the acute phase of ischemia. In this line, a very elegant perspective by Lo2 has recently proposed that, because most molecular targets for therapy have biphasic roles in stroke pathophysiology, failure in neuroprotection may partly be due to the fact that many neuroprotectants inhibit not only mechanisms of damage, but also those mechanisms needed for repair. This interesting hypothesis leads to propose that new drugs for acute stroke treatment should be able to promote a “safe” neuroprotection with the ability to preserve those mediators required for neural repair.

Citicoline: A Drug for ‘Safe’ Neuroprotection Toward Repair
Cytidine-5-diphosphocholine (citicoline or CDP-choline) is a compound normally present in all cells and an intermediate in the biosynthesis of phosphatidylcholine. Citicoline has been shown to produce neuroprotective effects in a variety of central nervous system injury models, including cerebral ischemia. At the experimental level, it has been reported to decrease infarct volume and edema and/or to improve neurological deficits either alone or in combination with other agents (for review, see Adibhatla and Hatcher3).

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S33
In humans, citicoline is the only neuroprotectant that has shown positive results in all randomized, double-blind trials and has demonstrated efficacy in a meta-analysis with an overall safety similar to placebo. A large (n=2600) trial (ICTUS: International Citicoline Trial on Acute Stroke; Clinicaltrials.gov; October 31, 2006; www.clinicaltrials.gov/ct/show/NCT00331890ICTUS) is now enrolling patients with acute ischemic stroke within 24 hours of symptom onset in an effort to lend support to the results of the meta-analysis.

The effects proposed to explain some of the neuroprotective actions of citicoline have been thoroughly reviewed and include prevention of fatty acid release, stimulation of PtdCho synthesis, preservation of cardiolipin and sphingomyelin levels, increase of glutathione synthesis and glutathione reductase activity, restoration of Na\(^+\)/K\(^+\)-ATPase activity, and antiapoptotic effects. This wide range of actions suggests that its precise mechanism of action is not fully known yet.

**Citicoline in Neuroprotection**

Data from our laboratory have demonstrated that antixcitotoxic actions are involved in the neuroprotective actions of citicoline. Indeed, we found that citicoline is able to reduce infarct volume in parallel to a decrease in ischemia-induced elevation in brain glutamate levels measured by microdialysis. High extracellular glutamate concentrations in the brain may result from increased release and/or from decreased uptake. Ischemia-induced glutamate release has been shown to be largely due to reversed operation of neuronal glutamate transporters, a fact that results from a severe depletion in adenosine 5’-triphosphate levels caused by ischemia. Of note, our results also showed that ischemia-induced adenosine 5’-triphosphate loss was decreased both in vivo and in vitro by previous administration of citicoline. These results support the hypothesis that citicoline-induced effect on neuronal adenosine 5’-triphosphate levels accounts, at least in part, for the decrease in extracellular glutamate and the subsequent neuroprotection after ischemia very likely by keeping the normal function of neuronal transporters.

Interestingly, astrocytes play an important role in the maintenance of extracellular glutamate concentrations by regulating its uptake; glutamate transporters remove this neurotransmitter/excitotoxin from extracellular space thereby helping to terminate glutamatergic synaptic transmission and to prevent the extracellular glutamate concentration from rising to neurotoxic values. Using cultured rat astrocytes, we found that citicoline enhances glutamate uptake in these cells. Of the 5 high-affinity, sodium-dependent glutamate transporters currently known, 2 of them, EAAT1 and EAAT2, are localized primarily in astrocytes. In our experiments, citicoline remarkably increased astrocytic membrane levels of the EAAT2 transporter by inducing its translocation from the cytosol to the membrane, where it is functional and helps to decrease extracellular glutamate concentrations.

Then, the neuroprotective, antixcitotoxic effect of citicoline results from a dual action on both neurons and astrocytes: increased adenosine 5’-triphosphate levels in neurons, either from an increased production or from decreased consumption, may delay the reversal of neuronal glutamate transporters that leads to glutamate release and subsequent excitotoxicity; in addition, an increased capacity of astrocytes to remove excess extracellular glutamate would contribute to decrease ischemic damage.

In a subsequent study we demonstrated that citicoline induces a specific relocalization of EAAT2 into rat brain lipid raft microdomains, specialized membrane microdomains enriched in cholesterol and glycosphingolipids (for review, see Simons and Toomre) that serve as platforms for a variety of cellular functions such as vesicular trafficking and signal transduction, transmembrane signaling, lipid and protein sorting, viral uptake, and regulated proteolysis. The relocalization of EAAT2 into lipid rafts caused by citicoline may represent a new and efficient mechanism of regulation of glutamate transporter activity and subsequent management of extracellular glutamate levels because (1) EAAT2 is more strongly associated with lipid rafts than other glutamate transporters expressed in the brain; and (2) this association is important for the trafficking of EAAT2 to the lipid rafts in which glutamate uptake is more efficient. Importantly, increased EAAT2 association to lipid rafts by citicoline is associated with an increased function, as shown by improved glutamate uptake by lipid raft-associated plasma membrane vesicles. Interestingly, this phenomenon also takes place in vivo, where late administration of citicoline, 4 hours after the onset of the ischemic injury, is able to increase the EAAT2 association to lipid raft fractions, concomitantly to a reduction in infarct size, strongly supporting this mechanism as a mediator of neuroprotection in ischemic stroke.

Several studies have shown that EAAT2 deletion is related to larger increases in extracellular glutamate, neuronal damage, and brain edema after experimental brain ischemia. Interestingly, we reported that a polymorphism in the EAAT2 transporter promoter associates with higher and sustained plasma glutamate concentrations and increased frequency of neurological deterioration in patients with stroke, underscoring the importance of EAAT2 as a pharmacological target in the ischemic scenario. Although only experimentally a few drugs (estrogen, β-lactam antibiotics, glucocorticoid, and peroxisome proliferator-activated receptor-γ agonists) are known to increase EAAT2 expression, our present findings support the use of citicoline with this purpose not only in stroke, but also in other neurological disorders in which an altered function or expression of EAAT2 has been described such as epilepsy, Alzheimer disease, Huntington disease, and amyotrophic lateral sclerosis, among others.

**Citicoline in Neurorepair**

Stroke is a leading cause of death and long-lasting disability with a high socioeconomic burden; among 30-day survivors of first-ever stroke, approximately half survive for 5 years; of survivors, one third remain disabled, and 1 in 7 are in permanent institutional care. Taking into account that hemiparesis is the most common cause of disability after stroke, it seems essential to develop effective therapies to improve the motor recovery of these patients.

Due to its pleiotropic actions, we investigated whether chronic treatment with citicoline starting 24 hours after...
ischemia onset might affect stroke recovery and neuronal plasticity after focal ischemic injury in the rat. Our results showed that citicoline improves both sensorimotor integration and asymmetrical motor behavior after experimental stroke as determined by using the staircase skilled reaching test and the elevated body swing test. Interestingly, this was found in concordance with an increase in both dendritic complexity and spine density of pyramidal neurons of Layer V in the sensorimotor cortex contralateral to the insult in the group of animals treated with citicoline. The neurons studied include those that project to the forelimb region of striatum—among Layer V pyramidal cells, >40% project into dorsal-ventral striatum—and others into the rat spinal cord. Therefore, citicoline is likely to contribute to forelimb movement recovery acting on its different components. In addition, it has been suggested that changes in dendritic complexity are implicated in sensorimotor recovery induced by drugs or by rehabilitative training after experimental stroke. Our results are in agreement with previous evidence in animals and humans in which large infarcts affecting multiple structures involve reorganization and compensatory recruitment of the undamaged contralesional hemisphere.

In summary, citicoline increases neuronal plasticity and contributes to sensorimotor function recovery after experimental stroke. In the present collection of reviews, other actions of citicoline in different aspects of stroke pathophysiology are described that further support the potential of this drug for recovery. Taking into account that citicoline has been extensively studied in volunteers and patients demonstrating that is a well-tolerated and safe drug, as opposed to other therapies assayed for recovery, all these results may possess important implications by enhancing the therapeutic options not only for neuroprotection, but also for rehabilitation and overcoming suffering of patients with stroke.

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