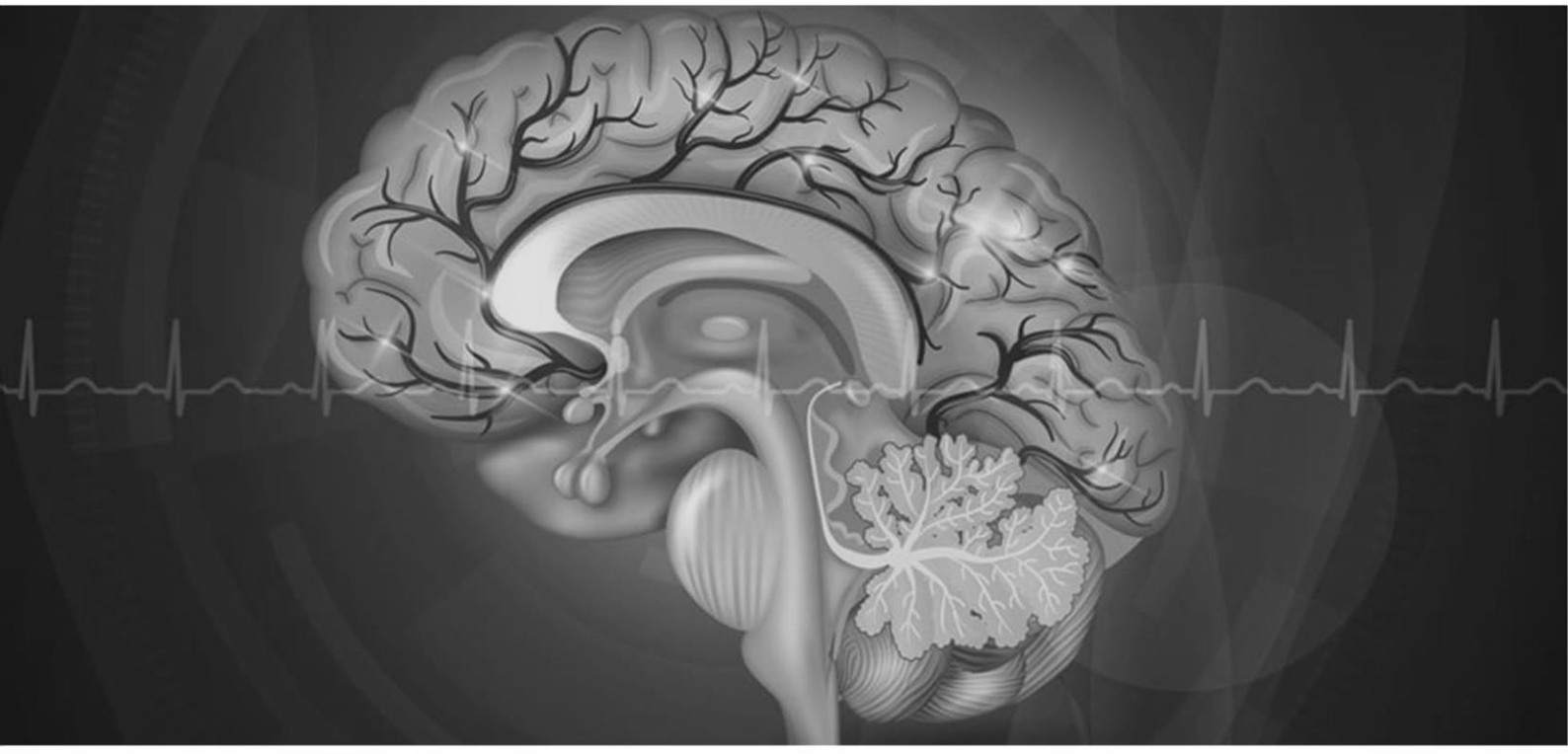
UNDERSTANDING THE USE OF CITICOLINE IN STROKE



Content

Background and Objective of the Survey

Citicoline, also known as cytidine diphosphate-choline, is a naturally occurring compound involved in the synthesis of phospholipids in cell membranes, particularly in the brain. Its neuroprotective properties and ability to enhance cellular repair and regeneration have led to its investigation as a potential therapeutic agent in stroke management.

In the context of stroke, citicoline is believed to exert its beneficial effects through multiple mechanisms. Firstly, it enhances the production of phospholipids, which are essential for cell membrane integrity and function, promoting neuronal repair and regeneration in the damaged brain tissue. Additionally, citicoline has been shown to increase the levels of neurotransmitters such as acetylcholine and dopamine, which play important roles in neuronal signaling and communication.

Furthermore, citicoline exhibits antioxidant and anti-inflammatory properties, reducing oxidative stress and inflammation in the brain following a stroke. By mitigating secondary injury processes, citicoline may help minimize neuronal damage and improve functional outcomes in stroke patients.

Clinical studies evaluating the use of citicoline in stroke have yielded mixed results. While some trials have reported positive effects on functional recovery, cognitive function, and neurological deficits, others have failed to demonstrate significant benefits compared to placebo or standard therapy. The variability in study outcomes may be attributed to differences in study design, patient populations, treatment regimens, and timing of citicoline administration relative to the onset of stroke symptoms.

The objective of the survey is:

To understand the use of citicoline in stroke

Methodology of the Survey

A survey was conducted to understand the use of citicoline in stroke. A total of 150 doctors from India participated in the survey.

Step 1: A literature search was done on the topic. Below topics were covered in the literature search

- Introduction
- Ischemic Neuroprotection: Brain Protection
- Citicoline
- Pharmacological actions
- Cerebral hypoxia and ischemia
- Synaptic transmission, intracellular signalling systems and neurotransmitter levels
- Learning performance, memory, and brain aging
- Citicoline Neuroprotection in Experimental Stroke
- Clinical Experience with Citicoline in Stroke Patients
- Neurorepair Therapies
- Citicoline and Brain Neurorepair
- Citicoline in Post-Stroke Cognitive Decline

Step 2: A survey questionnaire was prepared based on the literature search. The survey form was shared through the digital medium with physicians across India.

Step 3: Their responses were analyzed and the findings are provided in this survey analysis booklet.

Literature Review

Introduction[1]

Each year, about 22 million people worldwide suffer a stroke. Stroke is a global health-care problem that causes a substantial burden of disease and remains one of the most devastating public health problems, often resulting in death or severe physical impairment and disability. According to the *Global Burden of Disease Study 2010* in the last decade stroke became the third-most-common global cause of disability-adjusted life years (DALYs), second only to ischemic heart disease. Increase in vascular risk factors—in particular, high blood pressure, tobacco smoking, alcohol, and poor diets—appears to be responsible for this increase. Although effective primary prevention can be achieved with measures controlling vascular risk factors, at present, there are only two effective evidence-based treatments for stroke: stroke unit care and thrombolysis with alteplase (recombinant tissue plasminogen activator, rtPA).

Ischemic stroke is a dynamic process whereby the longer the arterial occlusion persists the larger the infarct size becomes and the higher the risk of post-perfusion hemorrhage. The goal of ischemic stroke treatment is to reopen the occluded artery. The only treatment that has demonstrably been able to halt the dynamic process launched by the vessel occlusion is rtPA that increases five times the odds of early recanalization (in the first 6 h), resulting in a decrease in infarct size with better neurologic and functional outcome of the patient. Currently, intravenous fibrinolysis can be administered safely within the first 4.5 h following stroke onset; and even as late as 6 h when an arterial occlusion is demonstrated with presence of potentially salvageable tissue (ischemic penumbra). In these late cases, the results of fibrinolysis treatment are similar to those of earlier windows in terms of arterial recanalization, functional recovery and frequency of hemorrhagic transformation.

Intravenous thrombolysis can be reinforced with ultrasound-enhanced treatment or sonothrombolysis and ultrasound plus microbubbles. Arterial recanalization in acute stroke can also been achieved by interventional neurovascular treatments including combined *i.v.* thrombolysis plus intra-arterial rescue in cases refractory to *i.v.* rtPA thrombolysis. In acute

stroke patients where rtPA is contraindicated other therapeutic options include primary intra-arterial thrombolysis and/or mechanical thrombectomy.

A recent systematic review and meta-analysis comparing intra-arterial thrombolysis *vs.* standard treatment or intravenous thrombolysis in adults with acute ischemic stroke demonstrated a modest benefit of intra-arterial thrombolysis over standard treatment, although no clear benefit was found for intra-arterial thrombolysis over intravenous thrombolysis in acute ischemic stroke patients. However, there was an almost fourfold increase in risk of intracranial hemorrhage (RR = 3.90; 95% CI 1.41–10.76; $p = 0.006$) with intra-arterial thrombolysis. In a study conducted by Álvarez-Sabin and colleagues, diffusion-weighted magnetic resonance imaging (DW-MRI) was performed in a group of patients with acute ischemic stroke involving the middle cerebral artery (MCA) territory. Initial DW-MRI was obtained within 6 h after ictus and was repeated 36–48 h later; images demonstrated increased size of the lesions in 77% of the patients. However, lesion-size increase was significantly smaller in those treated with *i.v.* rtPA than in those untreated (57.7% *vs.* 234.7%).

Therefore, recanalization treatment only controls partially the biochemical and molecular events triggered by cerebral ischemia, indicating that other factors must be controlled; such factors include, but are not limited to, collateral blood flow, body temperature, hyperglycemia, and blood pressure fluctuations. Ideally, sufficient protection must be provided to the ischemic brain (neuroprotection) along with enhanced recovery of the damaged brain (neurorepair).

Finally, incorporating stroke unit care and thrombolysis into medical services is difficult and even impossible in many low- and middle-income countries—which have the greatest burden of stroke—because the required high levels of infrastructure, expertise, and resources are unavailable. Therefore, safe and effective neuroprotective drugs that could be given at medical services with limited resources would improve the outcome of millions of acute stroke patients.

Ischemic Neuroprotection: Brain Protection[1]

Ischemic neuroprotection (brain protection) may be defined as any strategy, or combination of strategies, that antagonizes, interrupts, or slows down the sequence of injurious biochemical and molecular events that, if left unchecked, eventually result in irreversible ischemic injury. Neuroprotection attempts to limit the brain damage produced by ischemia.

Experimental studies have demonstrated the complexity of the pathophysiology of stroke. Among others, it involves excitotoxicity mechanisms, oxidative stress damage, inflammatory pathways, ionic imbalances, apoptosis, and angiogenesis that are potential targets being evaluated in clinical trials. Although successful in experimental models, translation to bedside treatments has been disappointing and complicated by some of the following reasons:

1. There is a need to protect the entire neurovascular unit that comprises neurons, glia, pericytes and blood vessels. For many years the goal was to salvage neurons in the ischemic penumbra but recently it became clear that this goal is insufficient and that all the elements of the neurovascular unit must be rescued from ischemia.

2. Many of the potential targets have a biphasic cycle whereby the same mediator or molecule plays a different role under pathologic or physiological conditions. For instance, in the earliest phase of ischemic stroke the excitatory glutamate NMDA receptors become hyperactive and mediate cell death, but these same receptors are critical for neurogenesis and neuronal plasticity during the recovery phase of stroke. A similar mechanism occurs with metalloproteases that contribute to the breakdown of the blood brain barrier (BBB) enlarging the ischemic lesion but are critical also for angiogenesis during the rec

Therefore, better animal models are required to explore the complexity of acute ischemic stroke. The use of preclinical STAIR criteria provides adequate guidelines but even the strict adherence to these criteria does not predict clinical success.

Because of the above reasons, and despite the large number of neuroprotective agents that have been proposed to interrupt the ischemic cascade based on successful animal studies, most therapeutic clinical trials of these agents have yet to show consistent benefit. According to Sahota and Savitz the most promising interventions that provide acute neuroprotection tested in larger clinical trials include hypothermia, magnesium sulfate, citicoline, and albumin. The most promising therapies enhancing neurorecovery in the subacute phase of stroke include granulocyte colony stimulating factor, G-CSF, citicoline, and cell-based therapies. Of all the above agents and methods, only citicoline appears to provide both neuroprotection and enhanced neurorepair with remarkable absence of side effects.

Citicoline[2]

Citicoline is the generic name of the pharmaceutical substance that chemically is cytidine-5'-diphosphocholine (CDP-choline), which is identical to the natural intracellular precursor of phospholipid phosphatidylcholine. CDP-choline is a mononucleotide consisting of ribose, cytosine, pyrophosphate, and choline whose chemical structure corresponds to 2-oxy-4-aminopyrimidine. CDP-choline is involved as an essential intermediate in the synthesis of structural phospholipids of cell membranes and formation of this compound from phosphorylcholine is the rate-limiting step of this biosynthetic pathway. The CDP-choline cycle is integrated into a larger metabolic network and its interruption can affect the distribution of lipid-related metabolites in several other pathways. CDP-choline is also related to acetylcholine metabolism. Thus, administration of CDP-choline is an exogenous source of choline and cytidine. Choline participates in several relevant neurochemical processes. It is the precursor and metabolite of acetylcholine, plays a role in single-carbon metabolism and is an essential component of different membrane phospholipids. The cytidine fraction, once transformed in uridine, is used for DNA and RNA synthesis as well as for the synthesis of membrane constituents and glycosylation, also having an important effect on purinergic receptors.

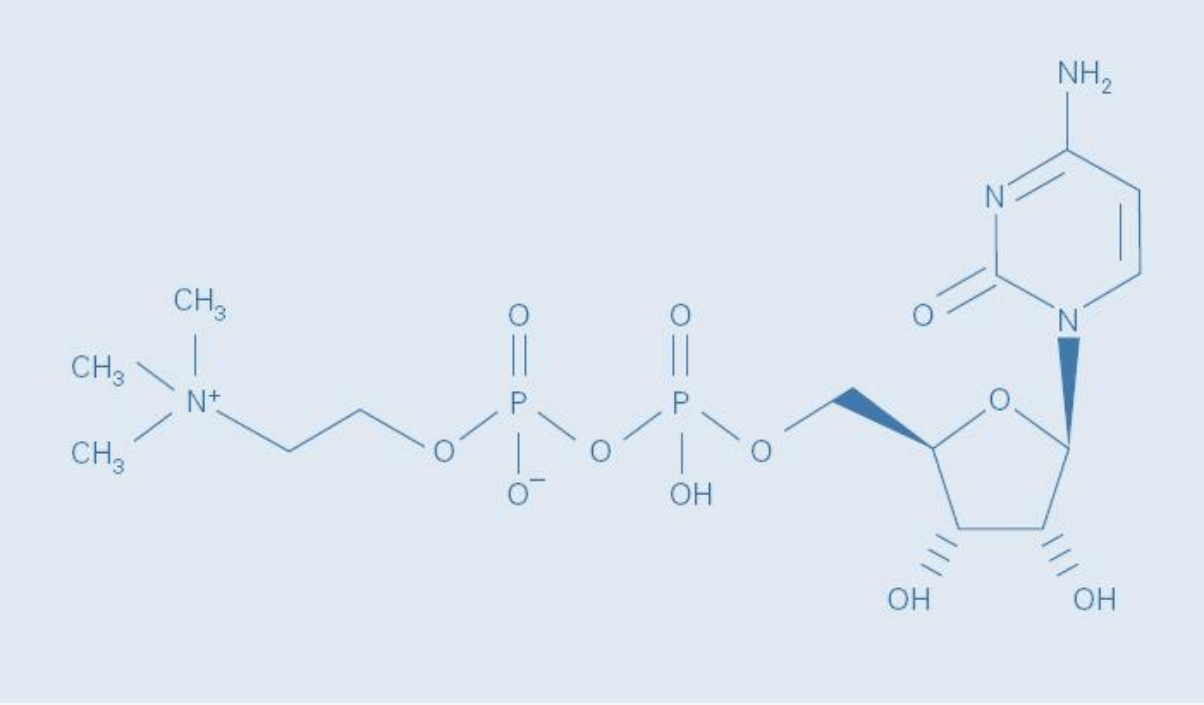


Figure 1. Chemical structure of CDP-choline (citicoline).

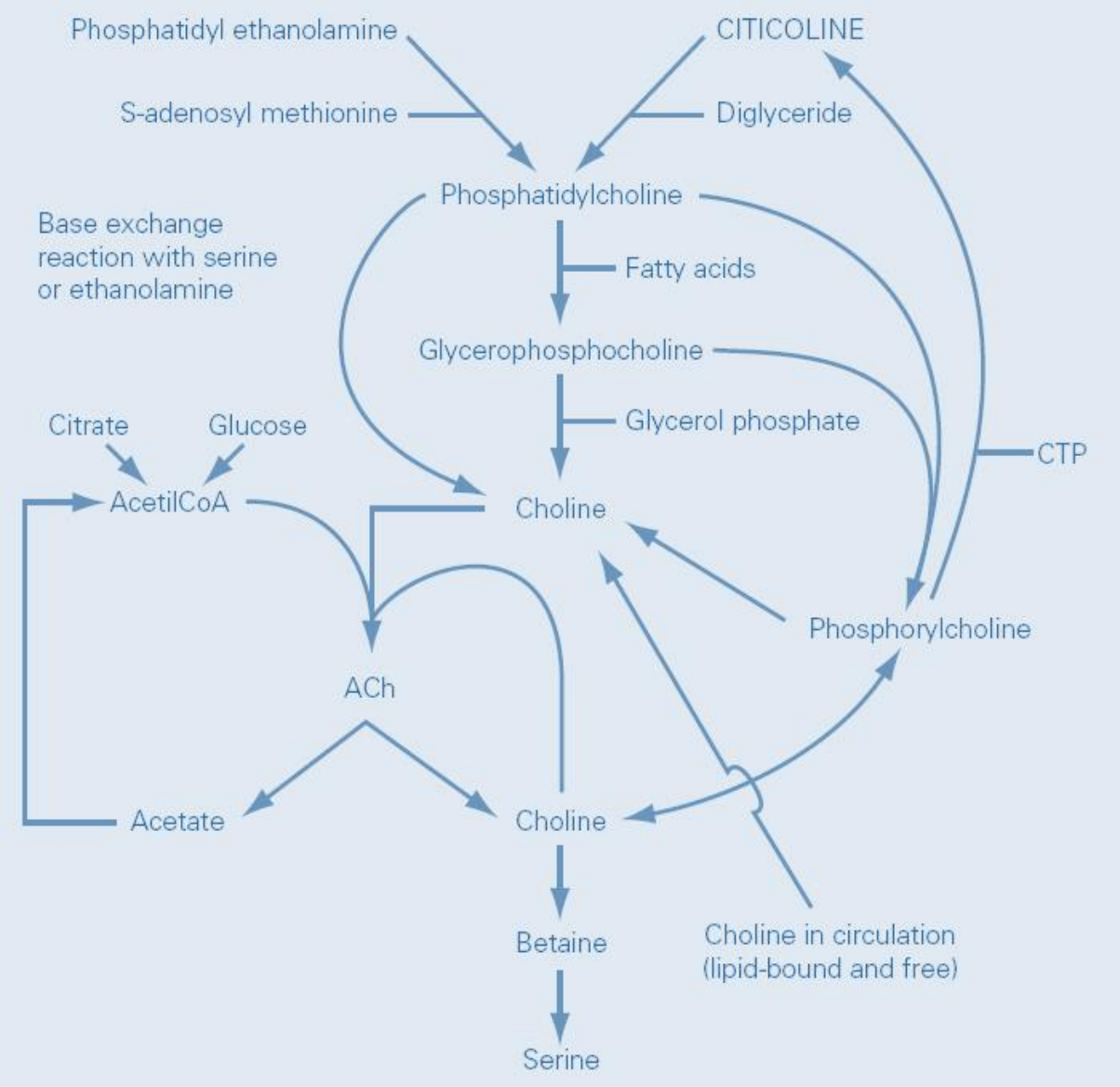


Figure 2. Relationship between citicoline and choline metabolism, cerebral phospholipids and acetylcholine.

Pharmacological actions[2]

Traumatic lesions and experimental cerebral edema

Javaid et al described the pathophysiological changes in brain phospholipids induced by traumatic brain injury, specially of choline-containing phospholipids such as phosphatidylcholine, and they highlight the role of choline-specific therapeutic strategies, such as the administration of citicoline, for the amelioration of traumatic brain injury.

Horrocks and Dorman have shown that citicoline and CDP-ethanolamine prevent degradation of choline and ethanolamine phospholipids during decapitation ischemia in rats and induce a partial reversion of free fatty acid release during reperfusion after experimental global ischemia in gerbils. Citicoline and CDP-ethanolamine, when administered together, have a synergistic effect and stimulate resynthesis of choline, ethanolamine, and inositol phospholipids, markedly decreasing free arachidonic acid levels.

In an experimental rat model of acute induced ischemia, LePoncin-Lafitte et al assessed integrity of the blood-brain barrier with labelled iodinated albumin, and brain metabolism using histoenzymological studies. In this experimental model, administration of citicoline was able to reduce vasogenic cerebral edema and to restore blood-brain barrier integrity. Authors also found that the size of induced infarctions was smaller with citicoline, and this compound decreased the activity of lactate dehydrogenase, succinyl dehydrogenase, monoamine oxidase, and acid phosphatase, emphasizing its protective role through a direct action at cell membrane level.

Mykita et al found in neuronal cultures that addition of citicoline after a hypocapnic lesion resulted in culture protection. Hypocapnia increases incorporation of labelled choline into phospholipids, while this process is slowed in the presence of citicoline. These authors concluded that citicoline is able to protect neurons under alkalosis conditions and may promote cell proliferation.

Yasuhara et al , in an electrophysiological study in rabbits, showed that citicoline decreased in the threshold for the arousal reaction and the threshold for muscle discharge, and concluded that this is a valuable drug for treatment of brain lesions because of its effects on consciousness and on the motor activity of the pyramidal system and its afferent pathways.

Martí Viaño et al compared the effects of pyriglutine, piracetam, centrophenoxine, and citicoline in a study on antagonism of barbiturate coma in mice. No differences were seen in animals treated with pyriglutine, piracetam, or centrophenoxine as compared to the control group, while with citicoline both coma duration and depth, as well as respiratory depression, were decreased as compared to all other groups. Arousal effects of citicoline were found to be due to increased cerebral blood flow, improved O_2 cerebral uptake and utilization of energy metabolism, and enhanced mitochondrial breathing.

Ogashiwa et al, in an experimental model of head injury in monkeys, established a significant dose-effect relationship between citicoline dose and coma duration, that started to be significant at doses of 60 mg/kg ($p < 0.05$).

Watanabe et al, studying the effects of several activators of brain metabolism, found that citicoline increased glucose incorporation and metabolism and decreased lactate accumulation in the brain, and also induced a slight increase of cerebral blood flow.

Alberghina and Giuffrida, in a study on nerve tissue response to a contusion lesion, showed that a moderate increase occurred in the activity of cholinephosphotransferase and was associated to a greater increase in the activity of phospholipase A_2 and several lysosomal hydrolases. They also found an increased number and size of lysosomes during neuronal regeneration. Arrigoni et al have shown citicoline to be able to completely inhibit activation of phospholipase A_2 without altering cholinephosphotransferase activity. On the other hand, Freysz et al showed that, in addition to decreasing phospholipase A_1 and A_2 activity, citicoline decreases free fatty acid release under hypoxic conditions, thus adding a protecting effect to its activating capacity of phospholipid reconstruction. Massarelli et al also showed citicoline action upon phospholipase A_1 and agreed with all other authors in their conclusions. Kitazaki et al also showed the inhibitory effect of citicoline upon membrane-associated phospholipase A_2 in rat brain cortex. Based on these characteristics, citicoline has been considered a non-specific inhibitor of phospholipase A_2 at intracellular level.

Algate et al tested the effects of citicoline in an experimental model of epidural compression in anesthetized cats. They noted that animals treated with citicoline had a greater resistance to the effects of mechanic brain compression as compared to animals in the control group. They also found that respiratory and cardiovascular changes were less intense in treated animals and concluded that citicoline provides a significant protection against the lethality of epidural compression. These results agreed to those obtained by Hayaishi and Kondo who showed an improvement in the electroencephalogram tracing following administration of citicoline to cats undergoing experimental brain compression, and also in survival quality.

Tsuchida et al administered ^{3}H-citicoline by the intraperitoneal route to rats subjected to cerebral cryogenic lesion by dry ice application on the scalp and confirmed the presence of the labelled drug in brain parenchyma, particularly in the white matter, and above all in damaged areas.

Boismare conducted an experimental model of craniocervical trauma without direct blow ('whiplash') in order to assess the effects occurring upon central catecholamine levels and found increased dopamine levels and decreased norepinephrine levels in the brain following trauma. This type of lesion causes postural dysregulation of brain supply and behavioural and learning disorders, that are related to accelerated degradation of cerebral norepinephrine. In animals treated with citicoline, trauma did not change the levels of these amines. The author stressed the protective role of citicoline, due to this stabilizing effect of catecholamine brain levels.

Clendenon et al showed that the decrease in Mg^{++}-dependent ATPase activity in the mitochondrial and synaptosomal membrane occurring in traumatic lesions is prevented by citicoline administration.

Cohadon et al, in a series of studies on a model of cryogenic cerebral edema in rabbits, showed that treatment with citicoline 20 mg/kg/day:

- – Slowed the drop in enzymatic activity of mitochondrial ATPase.
- – Restored Na^+/K^+ ATPase activity.
- – Restored oligomycin-sensitive ATPase activity.
- – Accelerated cerebral edema reabsorption, with normal values achieved in the fourth day, while such levels were not reached until the tenth day with spontaneous resorption.

These authors stated that the beneficial activity of citicoline in cerebral edema occurred by two mechanisms: by restoring insertion of membrane enzymes and enhancing their activity, and by acting upon edema by reducing water imbibition of brain parenchyma.

Lafuente and Cervós-Navarro conducted a microgravimetric study in experimental cerebral edema induced by ultraviolet radiation in cats to assess the effect of citicoline in this situation. The results suggested an action of citicoline decreasing the amount of edema, enhancing fluid reabsorption and accelerating fluid drainage to ventricles, i.e., increasing cerebral compliance. Authors concluded that CDPamines are helpful to control tissue lesions related to increased free fatty acids and to restore cell energy metabolism by restarting the Na^+/K^+ pump.

Majem et al assessed the electroencephalogram changes occurring in rats when cryogenic edema is induced, and how such electroencephalogram changes were modified by citicoline administration. These authors noted a significant increase in the theta frequency band during

the awakening state, with decreased delta and slow alpha bands and a lesser interindividual scatter of the overall frequency bands, which resulted in a greater electrogenic cerebral stability. They concluded that citicoline protected from the effects of cryogenic cerebral edema.

Roda, in an experimental model of cryogenic cerebral edema, measured extravasation of Evans blue through the blood-brain barrier and fluorescein uptake by astrocytes and neurons, and found that citicoline administration significantly reduced both processes as compared to control animals, thus allowing to state that citicoline has a direct effect upon transmembrane transport of sodium, potassium, water, and proteins at both blood-brain barrier endothelial cell level and astrocyte and neuron level. Though the exact mechanism of this action is not completely understood, its effect appears to occur at two levels: on the interface separating capillaries from the neuroglia and on cell membranes. Citicoline reduces microvascular permeability during experimental endotoxemia and in early burn edema in rats. Farshad et al propose citicoline as a potential protective agent in a model of hepatic encephalopathy, a known cause of cerebral edema. They found that citicoline supplementation enhanced the animal's locomotor activity and improved brain tissue markers of oxidative stress, concluding that the effects of citicoline on oxidative stress markers could play a fundamental role in its neuroprotective properties.

Cerebral hypoxia and ischemia[2]

In vitro studies using nerve tissues have shown anoxia to induce a decrease in the synthesis of structural phospholipids that is time-dependent, i.e., the longer the hypoxia the stronger the impact upon neuronal phospholipids metabolism. Moreover, a decreased incorporation of marked precursors into phospholipids of neuronal subcellular fractions obtained from animals subjected to experimental hypoxia has also been shown. It is also known that, when cerebral ischemia is experimentally induced, glycerophospholipids in cell membranes are broken down by the action of different phospholipases, producing free fatty acids and arachidonic acid derivatives. With prolonged ischemia, induced aggression upon membranes becomes more intense and membranes lose their functions. Na+ and Ca2+ accumulate inside the cell, triggering the ischemic cascade and invariably leading to cell death.

Under ischemia conditions, with the attendant neuronal distress, endogenous CDP-choline synthesis is compromised because the cell, under such conditions, lacks the high-energy phosphate compounds necessary for this biosynthetic route.

Because of the importance of restoring neuronal activity following cerebral ischemia and based on the experimental data discussed, various authors have investigated the effects of citicoline administration in various experimental models of cerebral ischemia and/or hypoxia.

Boismare et al reported that treatment with citicoline 20 mg/kg by the intraperitoneal route in rats induced, during acute hypoxia, a decrease in vegetative responses, protection from conditioned avoidance responses, and stabilization of dopamine and norepinephrine brain levels. This same group found in dogs subjected to normobaric hypoxia increases in blood pressure, heart rate, cardiac output, and regional blood flows, while no changes occurred in total peripheral resistance. Administration of citicoline abolished these hemodynamic effects induced by acute hypoxia, suggesting that this action was correlated to a dopaminergic agonistic effect of the drug. In cats subjected to short periods of cerebral ischemia, these authors noted that a depression occurred in cortical evoked potentials. Such depression was attenuated by prior administration of citicoline by the intracarotid route. These authors think that the protective effects of citicoline are metabolic/biochemical rather than hemodynamic in origin, and do not rule out a direct action of the drug upon central dopaminergic structures.

Alberghina et al investigated the effect of citicoline upon incorporation of labelled precursors into cerebral phospholipids of guinea pigs subjected to hypoxia. A group of animals were given 100 mg/kg of citicoline by the intraperitoneal route. Ten minutes later, the labelled precursors [2-3H]glycerol and [1-14C]palmitate were administered by the intraventricular route. Another group of animals only received the precursors, and acted as control group. Investigators noted that, as compared to the control group, citicoline-treated animals showed an increase in specific radioactivity of total lipids and phospholipids in purified mitochondria obtained from brain hemispheres, cerebellum, and brain stem. In a subsequent study, this same investigating team showed citicoline to be able to counteract the effects of hypoxia upon incorporation of labelled precursors into RNA and proteins, particularly at nuclear and mitochondrial level.

Various experimental studies have shown citicoline to prevent fatty acid release during cerebral ischemia and hypoxia, and to increase synthesis of structural phospholipids. Horrocks et al, using an experimental model of global cerebral ischemia by decapitation, showed that administration of a mixture of citicoline and CDP-ethanolamine decreased free fatty acid

release and increased synthesis of the corresponding glycerophospholipids, suggesting an involvement of choline and ethanolamine phosphotransferases.

Synaptic transmission, intracellular signalling systems and neurotransmitter levels[2]

As previously discussed, citicoline exerts some of its effects through its action on the levels of certain neurotransmitters and on some intracellular signaling processes. This section will discuss these specific effects upon neurotransmission and on intracellular signaling processes. As will be seen below, most studies have focused on analyzing the effect of citicoline on central dopaminergic transmission and on nicotinic cholinergic neurotransmission.

Martinet et al conducted a study in which the effects of citicoline administration on norepinephrine, dopamine, and serotonin levels were assessed in different rat brain regions. For this, conversion of ^{3}H-tyrosine and ^{3}H-tryptophan, administered by the intravenous route, into ^{3}H-norepinephrine, ^{3}H-dopamine, and ^{3}H-serotonin was measured, comparing the results obtained with administration of saline to those obtained after administration of citicoline at different doses. Metabolism of each neurotransmitter was studied in the brain regions where it has functional activity. Thus, for catecholamines citicoline action was studied in the striate body, brain cortex, and midbrain, while for serotonin the hypothalamus was also studied. The synthesis rate of dopamine, norepinephrine, and serotonin was expressed as a conversion index equal to the ratio between the amount of labelled neurotransmitter per gram of brain (cpm/g) and the tyrosine or tryptophan-specific radioactivity (cpm/mmol) in brain. citicoline significantly increased dopamine levels and synthesis rate in the striate body, and the effect exerted on tyrosine levels was very similar. Norepinephrine levels were increased in cortex, but showed no changes from control in the brain stem. As regards effects on serotonin, the drug was seen to cause decreases in the levels and synthesis rate of this neurotransmitter in the brain stem and hypothalamus, and no changes were seen in the cortex or striate. According to these authors, increased dopamine synthesis could be attributed to an enhancing effect of citicoline upon tyrosine hydroxylase activity, the rate-limiting step in dopamine synthesis. This activation of tyrosine hydroxylase would lead to an inhibition of dopamine reuptake at the synapse, an action that has been shown in *ex vivo* studies. By contrast, the increase seen in dopamine synthesis does not appear to be related to increased levels of tyrosine, since this completely saturates tyrosine hydroxylase under physiological conditions. The effects of citicoline upon striatal dopamine synthesis are particularly interesting because changes in

dopamine synthesis by extrapyramidal dopaminergic neurons are in the origin of Parkinson's disease.

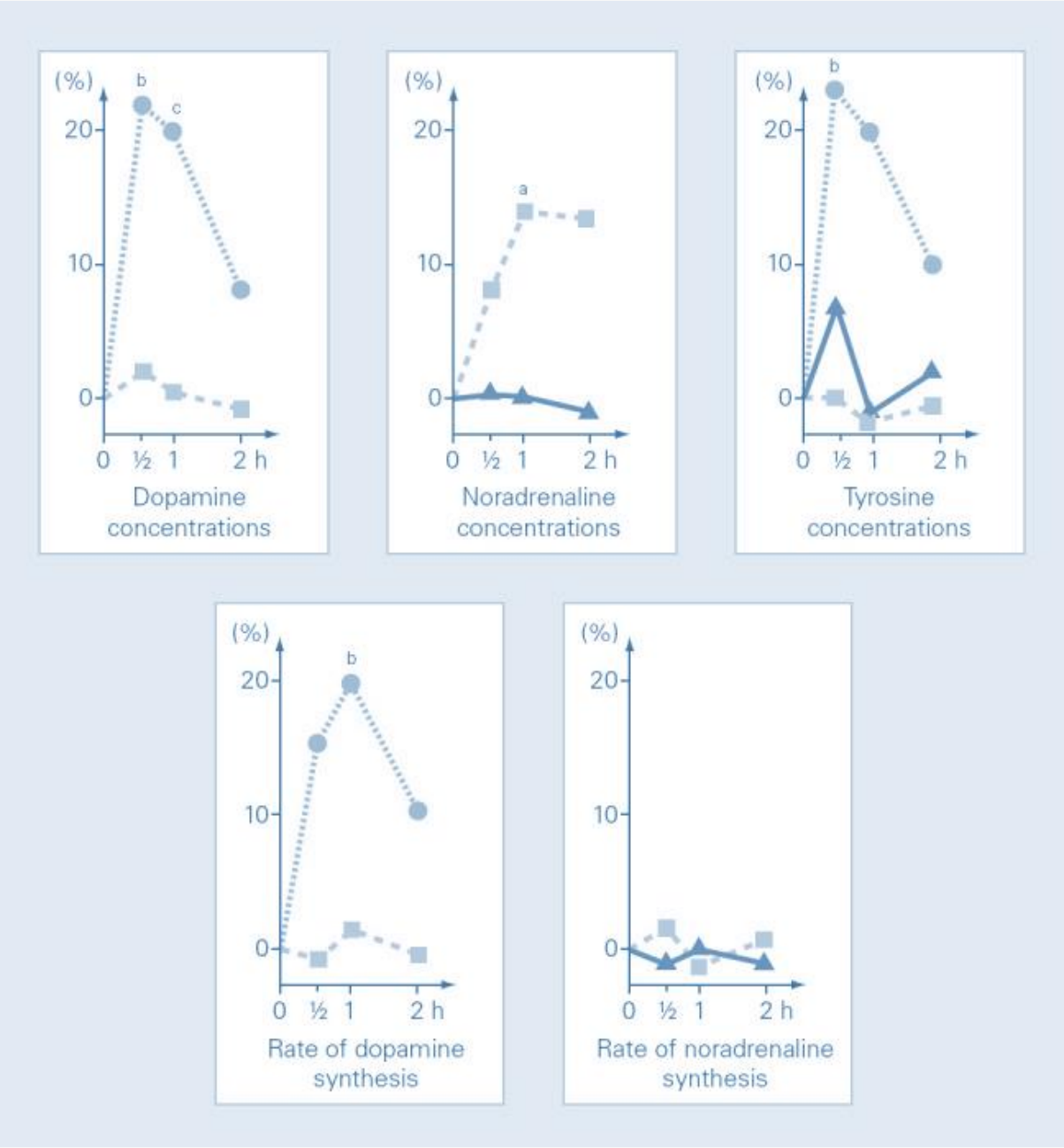


Figure 3. Influence of citicoline (30 mg/kg i.v.) on catecholamine synthesis at different time points after administration. The graphs show variations in catecholamine concentrations and rates of synthesis, in percentages with respect to the control, at different locations. • Corpus striatum; ■ Cortex; ▲ Brainstem-mesencephalon; [a] $p < 0.1$, [b] $p < 0.05$; [c] $p < 0.01$.

Saligaut et al obtained results in agreement with the previous ones when studying dopamine reuptake in synaptosomes taken from the striate body of rats previously treated with citicoline. Following long-term treatment with this drug, a decreased dopamine reuptake by synaptosomes was seen, and authors related this fact to the increase in tyrosine hydroxylase activity, that would involve an increased dopamine synthesis. They think that a structural change in neuronal membranes, mainly of phospholipid levels, could be one of the factors responsible for the change in synaptosomal reuptake of the neurotransmitter induced by citicoline. Hypobaric hypoxia was also seen to antagonize the inhibitory effect of citicoline on dopamine reuptake by synaptosomes. This antagonism may be explained by the fact that hypoxia decreases activity of tyrosine hydroxylase, an enzyme that requires oxygen, thus counteracting enzyme activation exerted by citicoline. This leads to a decreased dopamine synthesis and a subsequent increase in dopamine reuptake. These same authors studied citicoline action in the experimental oxotremorine-induced cholinergic syndrome in mice and showed that citicoline pretreatment does not potentiate this syndrome, but inhibits salivation induced by oxotremorine. Levodopa antagonized brain symptoms such as tremor-akinesia induced by oxotremorine. However, this antagonism disappeared in animals under long-term oral treatment with citicoline, thus confirming the action of citicoline on dopaminergic pathways. Citicoline effects appear to be mediated by hypersensitivity of some dopaminergic receptors, rather than by a direct stimulating effect on striatal dopaminergic receptors. In another series of experiments, these authors examined the effects of citicoline on catecholamine metabolism in the striate and hypothalamus from rats subjected to acute hypobaric hypoxia. The results show that citicoline partially counteracts the effects of hypoxia upon the release and metabolism of certain neurotransmitters. In another study, Saligaut et al analyzed the effects of citicoline in rats with unilateral nigrostriatal lesion induced by 6-hydroxydopamine. In damaged animals, amphetamine administration induced an ipsiversive circling behavior, while such circling behavior was contraversive with administration of levodopa and apomorphine. This appears to be mediated by the development in the damaged side of a supersensitivity of postsynaptic dopaminergic receptors. Subchronic treatment with citicoline did not induce behavioral effects. Citicoline did not change the stimulating effect of apomorphine, but potentiated the effects of levodopa and amphetamine. These data show that citicoline effects are mediated by a presynaptic mechanism. Although potentiation of levodopa may not be explained by an activation of tyrosine hydroxylase, this effect appears to be related to an improved release of dopamine synthetized from exogenous levodopa. Kashkin et al evaluate the effect on the combination of citicoline with levodopa/carbidopa in the rotenone model of Parkinson's

disease in rats, confirming that the combination therapy had more pronounced therapeutic effect on extrapyramidal disorders than monotherapy.

Cansev et al found that peripheral administration of citicoline increases plasma adrenaline and noradrenaline concentrations. Also, CDP-choline modulates monoaminergic and cholinergic transporters in rat brain.

Agut et al indirectly studied the effect of citicoline upon dopamine synthesis in the striate body by measuring local levels of dopamine metabolites in animals in which blockade of dopaminergic receptors had been induced by administration of haloperidol. Pretreatment with citicoline 100 mg/kg/day/5 days significantly increased levels of homovanillic acid and 3,4-dihydroxyphenylacetic acid in the striate of treated animals as compared to the control group. Increase in levels of these metabolites was even stronger in a group of animals also receiving apomorphine. Results obtained in this study suggest that citicoline increases dopamine synthesis in the striate of rats in which activation of such synthesis has been experimentally induced by haloperidol administration. This same investigating team subsequently conducted a study to examine whether citicoline alone, without provoking an increased dopamine demand by dopaminergic receptors, caused an increased synthesis of this neurotransmitter, resulting in increased striatal levels of its main metabolites, homovanillic acid and 3,4-dihydroxyphenylacetic acid.

Learning performance, memory, and brain aging[2]

It has been shown that hypobaric hypoxia decreases learning performance in rats undergoing sound avoidance conditioning, and that this effect may be antagonized by pretreatment with apomorphine or other dopaminergic agonists. These effects of hypoxia appear in relation to an inhibition of metabolism of cerebral catecholamines that would be ultimately responsible for an understimulation of central postsynaptic dopaminergic receptors. Based on these assumptions, Saligaut and Boismare conducted a study on the effects of citicoline administration upon learning performance in rats subjected to hypobaric hypoxia. Under hypoxic conditions, citicoline was administered at 300 mg/kg/day for 12 days to a group of rats that underwent learning tests of a sound avoidance conditioning in the last five days of treatment. Effects seen in this group were compared to those seen in another group receiving apomorphine 0.5 mg/kg 30 minutes before each daily conditioning session and to those recorded in animals receiving both treatments. A group of animals acted as control and received

an ascorbic acid solution under the same experimental conditions. Citicoline partially restored learning performance. The same effect, but to a lesser extent, was seen with administration of apomorphine and with combined administration of both drugs. These results suggest that administration of citicoline counteracts, as with dopaminergic agonists, the effects of hypoxia. Previously we commented the protective effect of citicoline against the cognitive impairment induced by chronic cerebral hypoperfusion.

Drago et al administered citicoline 10-20 mg/kg/day intraperitoneal for 20 days to 24-month-old Sprague-Dawley male rats from a strain showing cognitive and motor deficits. The drug was also given to rats with behavioral changes induced by a single injection of scopolamine, a cholinergic antagonist, by prenatal exposure to methylazoxymethanol, or by bilateral injections of kainic acid into the magnocellular basal nuclei. In all cases, citicoline improved learning and memory performance, evaluated using active and passive avoidance tests. In the old rat group, improved motor capacity and coordination was also seen. For these authors, these results suggest that citicoline affects the central mechanisms involved in cognitive behavior, probably through a cholinergic action.

Citicoline Neuroprotection in Experimental Stroke[3]

Citicoline (cytidine-5′-diphosphocholine or CDP-choline) is a naturally occurring endogenous compound, originally identified by Kennedy in 1956 as the key intermediary in the biosynthesis of phosphatidylcholine. Citicoline is composed of two essential molecules, cytidine and choline, the structural phospholipids of cell membranes. Phospholipids are essential constituents of cells and have a high turnover rate, which requires the continuous synthesis of these compounds to ensure the adequate function of cell membranes. Damaged cell membranes and impaired metabolism of phospholipids have been implicated in the pathophysiology of cerebral ischemia. It appears that an important component of citicoline neuroprotective capacity is its ability to improve phosphatidylcholine synthesis in the injured brain.

A large number of research studies have explored the protective effects of citicoline in experimental stroke models. At the experimental level, citicoline has been reported to decrease infarct volume and to reduce brain edema, with improvement of neurologic deficits either as a single therapy or in combination with other agents, including rtPA and nimodipine. A large meta-analysis of experimental stroke studies with citicoline in ischemic stroke concluded that citicoline reduces infarct volume by 27.8% (19.9%–35.6% $p < 0.001$). However, as mentioned

later, its effects vary with the dose whereby higher doses of citicoline produced greater reduction of brain damage compared with lower doses. Using a recent experimental model of stereotactic drug delivery to bypass the BBB delivering citicoline in direct contact with ischemic neurons in a MCA occlusion model in rats, Xu *et al*. demonstrated optimal effects of citicoline administration by this stereotactic delivery method under MRI guidance.

Citicoline has therapeutic effects at several stages of the ischemic cascade in acute ischemic stroke. First, it stabilizes cell membranes by increasing phosphatidylcholine and sphingomyelin synthesis and by inhibiting the release of free fatty acids. By protecting membranes, citicoline inhibits glutamate release during ischemia. In an experimental model of ischemia in the rat, citicoline treatment decreased glutamate levels and stroke size. Caspase is activated in human stroke and citicoline has been shown to decrease the release of damaging caspase activation products inhibiting apoptosis in animal models of brain ischemia. Citicoline favors the synthesis of nucleic acids, proteins, acetylcholine and other neurotransmitters, and decreases free radical formation. Therefore, citicoline simultaneously inhibits different steps of the ischemic cascade protecting the injured tissue against early and delayed mechanisms responsible for ischemic brain injury. Finally, citicoline may facilitate recovery by enhancing synaptic outgrowth and increased neuroplasticity with decrease of neurologic deficits and improvement of behavioral performance, as well as learning and memory tasks.

Clinical Experience with Citicoline in Stroke Patients[3]

For the past two decades, multiple randomized clinical stroke trials on citicoline reported the effectiveness of this pharmacological intervention when used early after onset of ischemia, as demonstrated by improvements in level of consciousness and modified Rankin score. Given that various populations of stroke patients were included in these studies using different sample sizes, multiple doses, and several outcome endpoints, it became difficult to reach valid conclusions. Most studies, however, demonstrated a positive effect with the use of citicoline during the acute and subacute phases of ischemic stroke. For instance, the *ECCO 2000* trial included 90 patients that underwent diffusion-MRI prior to the onset of the treatment and a second one with T2 sequences 12 weeks later. Patients treated with 2 g daily of citicoline orally had an initial lesion volume of 62 mL and this was reduced six weeks later to 17 mL; in comparison with controls, the MRI reduction in infarct size was statistically significant. Moreover, 70% of the patients with clinical improvement of greater than seven points in the

NIH stroke scale had smaller stroke size compared with 42% in those without clinical improvement.

Data Pooling Analyses[3]

In 2002, we performed a data pooling analysis to determine the effect of citicoline on neurological and functional recovery three months after moderate to severe stroke (baseline NIH ≥ 8) in comparison with placebo. The main outcome measure was global improvement using Generalized Estimating Equations (GEE analysis), *i.e.*, the degree of neurological and functional recovery represented by the global scores of the NIH Stroke Scale (NIH-SS ≤ 1), Barthel's Index (BI ≥ 95%) and the modified Rankin score (mRS ≤ 1). This study reviewed all randomized double-blind, parallel, placebo-controlled studies performed in patients with ischemic stroke treated with either citicoline or placebo within the first 24 h of the onset of symptoms and during a period of six weeks. The daily oral doses used ranged from 500 mg, 1000 mg, to 2000 mg. The patients included fulfilled the following criteria: age ≥ 18 years, randomized within the first 24 h after onset of stroke symptoms, persistent deficits for >60 min, brain CT and/or MRI compatible with the diagnosis of stroke, symptoms suggestive of acute ischemia in the MCA territory, baseline NIH score ≥ 8 (with at least two points from motor deficit), and mRS ≤ 1 prior to the stroke. Finally, subjects had none of the following exclusion criteria: brain CT/MRI with other structural lesions, serious systemic disease, unstable cardiovascular disease, pre-existing disability and/or psychiatric disease or dementia.

Following a comprehensive review, a total of 1372 patients were included in the data pooling analysis, 789 treated with citicoline and 583 with placebo, from four controlled clinical trials performed in the USA. After 12 weeks of treatment 25.2% of the patients treated with citicoline presented complete recovery compared with only 20.2% of the placebo-treated cases (OR 1.33; 95% CI 1.10–1.62; $p = 0.0034$). As mentioned above, patients included in the data pooling analysis received three different daily doses of citicoline: 500 mg, 1000 mg or 2000 mg; the group treated with 2000 mg/day had statistically significant better prognosis with a 38% higher probability of complete recovery at 12 weeks compared with those at lower doses.

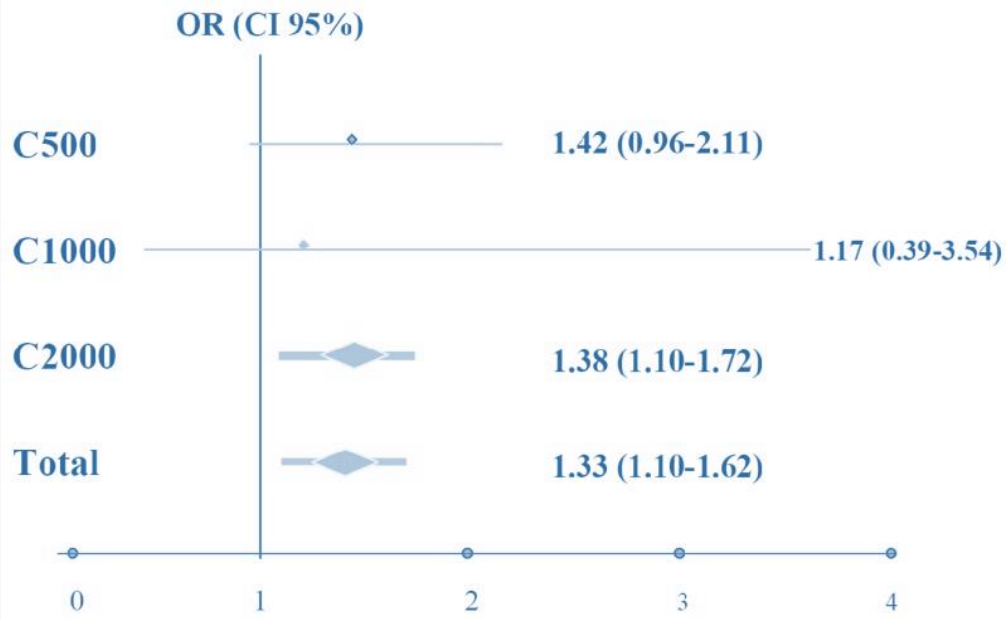


Figure 4. Probability of total recovery according to daily dose of citicoline among patients included in the pooled data analysis (*N* = 789 subjects on citicoline compared with 583 on placebo).

Upon individual analysis of each one of the three variables that conform to the main global variable, it was determined that improvement occurred both with neurological deficits measured by the NIH-SS, as well as with functional scales (BI and mRS). In comparison with placebo-treated subjects, citicoline-treated patients reached a higher percentage of complete neurological and functional recovery. This was particularly clear with mRS scores (OR 1.42; 95% CI 1.08–1.88; $p = 0.013$). There were no differences in side effects or number of cases withdrawing from the trial between the two groups.

In summary, the results of the data pooling analysis concluded that patients with moderate to severe ischemic stroke (NIH ≥ 8) treated with citicoline orally within 24 h of onset for a period of six weeks demonstrated a statistically significant increase of 33% in the probability of achieving complete recovery at 12 weeks; furthermore, it was demonstrated that citicoline is a safe medication.

A meta-analysis by Sever of 10 controlled clinical trials using citicoline studied 2279 patients, including both ischemic and hemorrhagic stroke distributed as follows, ischemic stroke: 1278 (1171 on citicoline *vs*. 892 controls) and 215 intracerebral hemorrhages (107 on citicoline *vs*. 109 controls). This meta-analysis demonstrated similar results to those of the data pooling

analysis. In comparison with placebo, patients treated with citicoline showed significant reduction in the frequency of death or disability at follow-up (57.0% *vs*. 67.5%; OR 0.64; 95% CI 0.54–0.77; $p < 0.001$). Safety analysis showed no adverse effects in comparison with placebo (14.5% *vs*. 14.0%; OR 0.99; 95% CI 0.77–1.21; $p = 0.94$).

The ICTUS Trial[3]

The International Citicoline Trial on Acute Stroke, ICTUS was designed to confirm the encouraging results of the data pooling analyses and to replicate those trends. ICTUS was an international, multicenter, prospective, double-blind, randomized, placebo-controlled trial with participation of neurology services from 37 centers in Spain, 11 in Portugal, and 11 in Germany. Patients were randomized in a 1:1 ratio to citicoline or placebo. Citicoline was dosed at 2000 mg/day during six weeks; in the first three days it was given intravenously (1000 mg/12 h) and orally from the 4th day on for six weeks (two tablets 500 mg/12 h).

The main objective of the study was to confirm the results of the data pooling analysis; *i.e.*, to determine the overall effects of citicoline on moderate to severe ischemic stroke recovery (NIHSS at baseline ≥8) after three months of therapy with 2000 mg/day of citicoline (six weeks of treatment and 6 weeks of follow-up) in comparison with placebo. The global variable previously used in the data pooling analysis was the main end-point, with three components: neurological deficit (NIH-SS ≤ 1), functional capacity (mRS ≤ 1) and activities of daily living (BI ≥ 95). The main global variable was studied using GEE analysis.

The results were as follows: from a total of 2298 patients enrolled into the study 1148 were assigned to citicoline and 1150 to placebo. The trial was stopped for futility at the 3rd interim analysis on the basis of complete data from 2078 patients. Global recovery at 90 days was similar in both groups. The median unbiased estimate of the adjusted odds ratio of the primary efficacy endpoint was 1.03 (95% CI 0.86–1.25). The odds ratios were also neutral in the sub-groups defined by minimization factors. Similar results were reported for each one of the secondary objectives (mRS ≤ 1, NIHSS ≤ 1, Barthel index ≥95). Mortality was comparable between the two groups (19% in the citicoline group *vs*. 21% in the placebo group). Adverse events occurred with similar frequency in both groups. Symptomatic hemorrhagic transformation occurred in 6% of patients who received citicoline and 8% of patients assigned to placebo ($p = 0.25$).

Some important characteristics of the ICTUS trial probably influenced the results:

1. Patients had more severe strokes in the ICTUS trial, as demonstrated by the NIH-SS 15 *vs*. 14 in previous studies; this renders more difficult the demonstration of a favorable effect; the main end-point required global improvement of both neurological and functional measurements. In fact, in the ICTUS trial the mRs 0–2 was 29% *vs*. 39% for pooled cases.

2. It is conceivable that larger doses for a longer period could have had a positive effect. In the previously noted meta-analysis of experimental data greater reduction of infarct volume occurred in rats treated with larger doses of citicoline (300–500 mg/kg), along with superior recovery (27%; 95% CI 9–46) in comparison with animals treated with lower doses (100–300 mg/kg) with 18% recovery (95% CI 5–32; $p > 0.001$). Larger reduction of stroke volume was also documented in another study; moreover, citicoline at high doses is as effective as *i.v*. thrombolysis in experimental stroke.

3. Patients enrolled in the ICTUS trial were not required to have neuroimaging studies of ischemic penumbra. Therefore, it was impossible to determine if at the onset of therapy salvageable brain tissue was present; moreover, this lack of images prevented accurate evaluation of stroke evolution. The latter is highly relevant given that in the *ECCO 2000 Citicoline Trial—DWI Sub-study* a comparison of DW-MRIs obtained at baseline with T2 MR images at week 12 of treatment with citicoline (2 g/day for six weeks) showed a significant decrease in volume of the cortical lesion; this reduction in lesions size was associated with better clinical outcome, as mentioned above.

4. Finally, a substantial number of patients received *i.v*. rtPA rendering the analysis of the results more difficult since many patients reached the maximum possible recovery with the thrombolytic treatment. Thus, a *ceiling effect* resulting from an already maximal improvement due to rtPA effect cannot be ruled out. Almost half of the patients (47%) in the ICTUS trial received *i.v*. rtPA compared with only 13% in the pooled data analyses. Additionally, the trials were done 10 years apart, a period of time during which the standard of stroke care has improved substantially.

Hemorrhagic Stroke[3]

A single clinical trial (*FI-CDPc-HIC*) has used citicoline in patients with hemorrhagic stroke. This was a pilot, double-blind, randomized, placebo-controlled trial to evaluate the efficacy and safety of citicoline in patients with acute intracerebral hemorrhage (AICH). The study enrolled patients aged 40–85 years old with a primary hemispheric supratentorial hemorrhage within less than 6 h of evolution. Patients were treated with placebo or citicoline 1 g/12 h *i.v.* during the first week and then orally. Safety analysis showed no differences with placebo in terms of adverse effects, mortality or study withdrawals. The results showed that 6.7% of the patients treated with placebo had reached independence (Rankin 0–2) at 12 weeks compared with 27.8% of those on citicoline. In conclusion, citicoline is a safe and effective pharmacological product in patients with AICH and can be used in acute stroke patients even before images are obtained to separate ischemic from hemorrhagic stroke.

Brain Neurorepair[3]

Spontaneous recovery of function occurs naturally after stroke in both humans and in animal models. This functional recovery is generally incomplete and results from reversal of diaschisis, activation of cellular genesis, repair mechanisms, change in the properties of the existing neuronal pathways and stimulation of neuronal plasticity leading to new neuronal connections.

In patients with ischemic stroke neurological recovery occurs over a period of three months, and this is the usual evaluation time for final outcome in neuroprotection trials. However, recovery is only possible when neurorepair occurs, including not only repair of the damaged neurons, but also enhancement of angiogenesis and brain plasticity (neuronal and synaptic).

The adult human brain has the capacity to undergo physiological and anatomical modifications leading to motor and cognitive recovery. Cerebral ischemia launches concurrently neurogenesis and angiogenesis, two closely interconnected processes that enhance neural repair.

There is definitive evidence that neurogenesis occurs in the adult brain following a stroke. Endogenous progenitor neural stem cells are normally present in the normal brain and maintain the capacity to produce new neurons and glial cells during adult life. Progenitor neural stem cells capable of producing neuroblasts in the adult human brain are situated in the

subventricular zone of the lateral ventricle and in the dentate gyrus of the hippocampus. Under physiological conditions the neuroblasts of the subventricular zone migrate towards the olfactory bulb where they are transformed into neurons. In response to brain ischemia, the adult progenitor neural cells proliferate in the ipsilateral subventricular zone and migrate towards the zone surrounding the infarction where they mature into adult neurons that may become part of functional neuronal circuits.

Neuropathological studies have shown the increase in cellular proliferation and in neuroblasts in the subventricular zone in patients who died shortly after an acute ischemic stroke. However, many of the newly formed immature neurons and neural cells die and are never integrated into functional neuronal circuits. For this reason, it is important to develop novel cellular and pharmacological strategies to increase neurogenesis leading to functional neuronal circuits. Repair of focal cortical strokes is not done by neuroblasts migrating from the subventricular zone but from clonal neural spheres originating from the peri-infarct area that differentiate into neurons, astrocytes, oligodendrocytes, and smooth muscle cells.

Angiogenesis is one of the main components of the processes of post-ictal neurovascular remodeling. It induces capillary neoformation in response to proliferation and migration of primordial stem cells originating from the existing blood vessels. The pericytes appear to have a major role in neurogeneration responses. The pericyte is a pluripotent stem cell in the brain with the potential of differentiating into cells of neural lineage such as astrocytes, oligodendrocytes and neurons. Angiogenesis can be observed several days following an ischemic stroke and it has been shown that a higher capillary density correlates with longer survival. Proangiogeneic factors such as vascular endothelial growth factor or VEGF, and metalloproteinases increase following cerebral ischemia. The effect of angiogenesis is to increase collateral circulation to meet the metabolic demands in terms of oxygen, glucose and nutrients required by the damaged and repaired tissues. Also, the newly generated blood vessels provide the neurotrophic support required by neurogenesis and synaptogenesis that eventually lead to functional recovery. In summary, angiogenesis provides the stimulation required to launch and enhance endogenous mechanisms repair and recovery including neurogenesis and synaptogenesis, as well as neuronal and synaptic plasticity. These events are all involved in the long-term repair and restoration process that take place in the brain after acute or chronic ischemic events; therefore, angiogenesis is one of the most promising areas of research in the field of stroke treatment.

Neurorepair Therapies[3]

Repair therapies aim to restore the brain, a goal that differs from that of neuroprotection therapies, in which the aim is to limit acute stroke injury. A number of potentially useful post-stroke interventions are currently being evaluated, such as the “mirror therapy” that is simple and useful to apply in addition to traditional physical therapy and rehabilitation treatments. Neuromuscular electrical stimulation has been found to improve neuromuscular function and to stimulate cerebral plasticity.

Transcranial magnetic stimulation, in addition to physical and occupational therapy, significantly improves motor function. Improvement is due to stronger stimulation of intact motor cortical regions homolateral to the hemiplegic side.

The NEST-3 trial is currently being conducted. This is a multicenter, double-blind, randomized, placebo-controlled pilot study with parallel groups to evaluate the safety and efficacy of a transcraneal laser stimulation with the NeuroThera® Laser System in patients within 24 h of an acute ischemic ictus. Finally, there is an enormous potential with the use of robotic therapy after stroke.

A number of medications have been used to enhance recovery and tissue repair following ischemic stroke. Among the anti-depressants, serotonine uptake inhibitors (SSRIs) and noradrenergic inhibitors have been demonstrated to improve motor recovery in patients with ischemic stroke. The mechanism of action of SSRIs is unknown. Acler and colleagues described decreased excitability of the threshold of the contralateral motor cortex after one month of use of citalopram. Decreased contralateral threshold increases motor recovery; neurogenesis and synaptic plasticity when the treatment is used for periods as long as one year. Valproic acid treatment appears to decrease stroke size in experimental stroke in rats, probably by enhancing angiogenesis in the hemisphere ipsilateral to the arterial occlusion.

Citicoline and Brain Neurorepair[3]

In addition to the neuroprotective effects, citicoline also possesses a substantial neuroregenerative potential that may explain better its long-term beneficial effects in post-stroke patients.

In an experimental stroke model with permanent occlusion of the distal MCA in mice citicoline (500 mg/kg) or vehicle was administered 24 h later intraperitoneally for 1–2 weeks. Citicoline treatment decreased neuronal apoptosis and promoted endogenous cerebral repair. A well-known experimental study conducted at Madrid's Complutense University demonstrated that treatment with citicoline 24 h after MCA occlusion in rats produced an increase in neuronal synaptic spines with increased motor and functional recovery in treated animals.

Endothelial progenitor cells (EPCs) are circulating immature pluripotential hematopoietic cells capable of differentiating into mature endothelial cells to help in the recovery of capillary and vascular recovery of ischemic areas. EPCs also promote growth factor release and increase neurogenesis. The increase in circulating EPCs after acute ischemic stroke is associated with good functional outcome, reduced infarct growth and neurological improvement. It has been shown that increase in EPCs in peripheral blood in acute stroke patients improves functional recovery and decrease stroke size. In a prospective study including 48 patients with a first-ever non-lacunar stroke citicoline treatment and the co-treatment with citicoline and rt-PA are independently associated with a higher increase in circulating EPCs during the first week in acute ischemic stroke. Gutiérrez-Fernández *et al*. demonstrated in an experimental model of stroke in rats that treatment with CDP-choline significantly improved functional recovery associated with a decrease in lesion volume by MRI, less cell death and decreased expression of low-density lipoprotein receptor-related protein (LRP). In fact, CDP-choline increased cell proliferation, vasculogenesis and synaptophysin levels and reduced glial fibrillary acidic protein (GFAP) levels in the peri-infarct area of the ischemic stroke. A more recent study on 40 rats treated at 24 h of experimental stroke with citicoline during 10 days showed significant improvement in both motor and somatosensory recovery by increasing neurogenesis in the peri-infarct area, subventricular zone and dentate gyrus.

In summary, citicoline enhances both brain neuroprotective and neurorepair mechanisms following ischemic stroke.

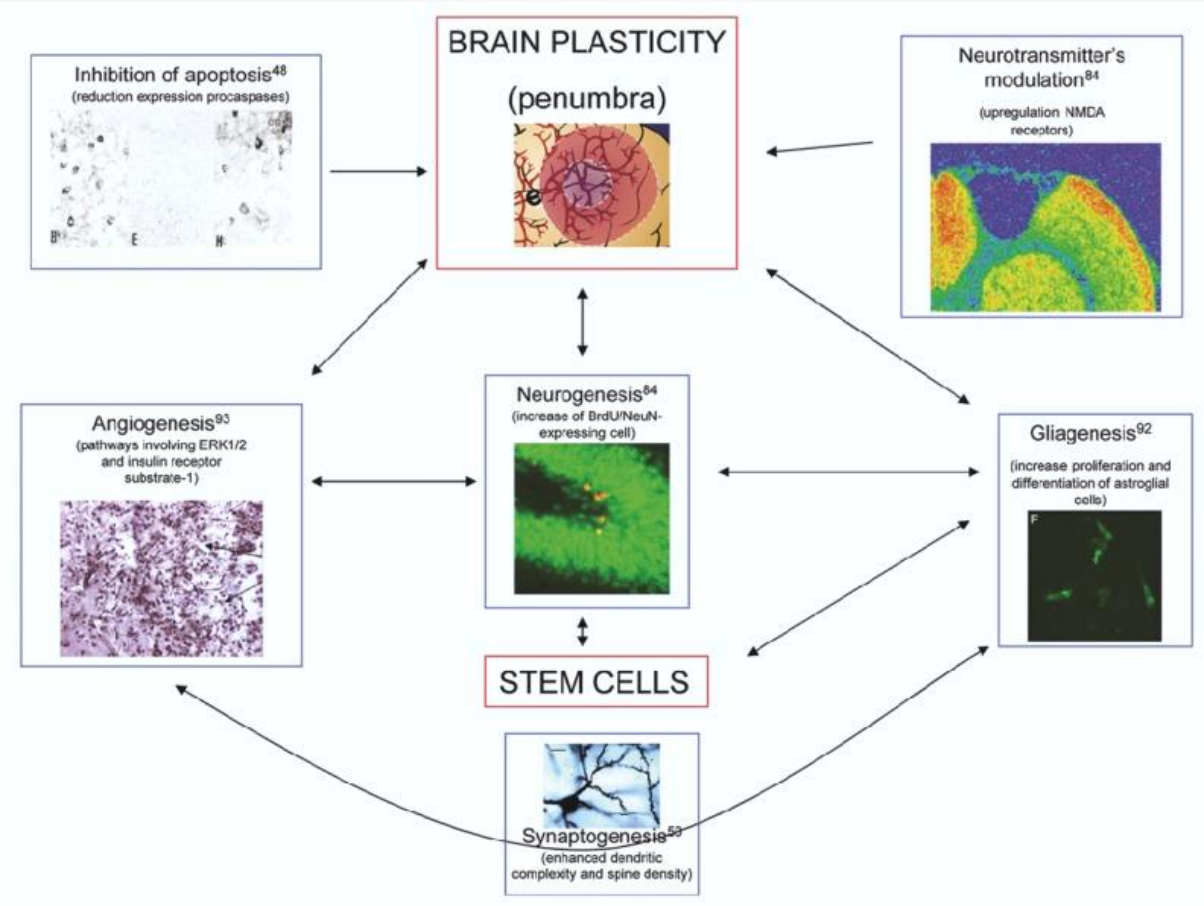


Figure 5. Major mechanisms involved in brain plasticity. The diagram explains the actions of citicoline to enhance the processes of inhibition of apoptosis, angiogenesis, neurogenesis, gliagenesis, synaptogenesis, and modulation of neurotransmitters. Notice that all these effects are similar to those induced by stem cells.

Citicoline in Post-Stroke Cognitive Decline[3]

Cognitive and behavioral manifestations are frequently observed in patients with vascular cognitive impairment and vascular dementia. Cognitive impairments occur in nearly half of stroke survivors, a frequency more elevated than that of stroke recurrence. These impairments may be more important determinants of functional outcomes after stroke than physical disability.

Most end-points used in clinical trials address issues relevant to motor function, activities of daily living and quality of life; in fact, many patients with cognitive or behavioral problems are excluded from clinical trials. Therefore, there is a need to identify cognitive and behavioral problems occurring as a result of stroke or "silent" small-vessel vascular disease. For the above

reasons, International Guidelines recommend routine cognitive and behavioral evaluation of stroke patients. In reality, these aspects are rarely evaluated in stroke patients. Along the same lines, few pharmacological products have been evaluated for prevention or treatment of cognitive problems in the stroke patient. A Cochrane meta-analysis of citicoline in 942 patients with vascular cognitive impairment studied in 12 placebo-controlled, double-blind, randomized studies showed modest evidence of improvement in memory and behavior, and a significant impression of improvement on the global impression of change on the part of caregivers. Based on these data and on abundant evidence on the neuroprotective and neurorepair effects of citicoline, we evaluated the safety and efficacy of citicoline on the cognitive manifestations of patients with acute ischemic stroke. This study was an open-label, randomized, parallel study of citicoline (1 g/day) for 12 months *vs*. usual treatment in patients with first-ever ischemic stroke. Citicoline-treated patients showed better outcome at follow-up in attention-executive functions and temporal orientation at six months and 12 months. Moreover, although differences are not statistically different, patients treated with citicoline showed a trend towards having a better functional outcome, measured with mRS at 6 and 12 months.

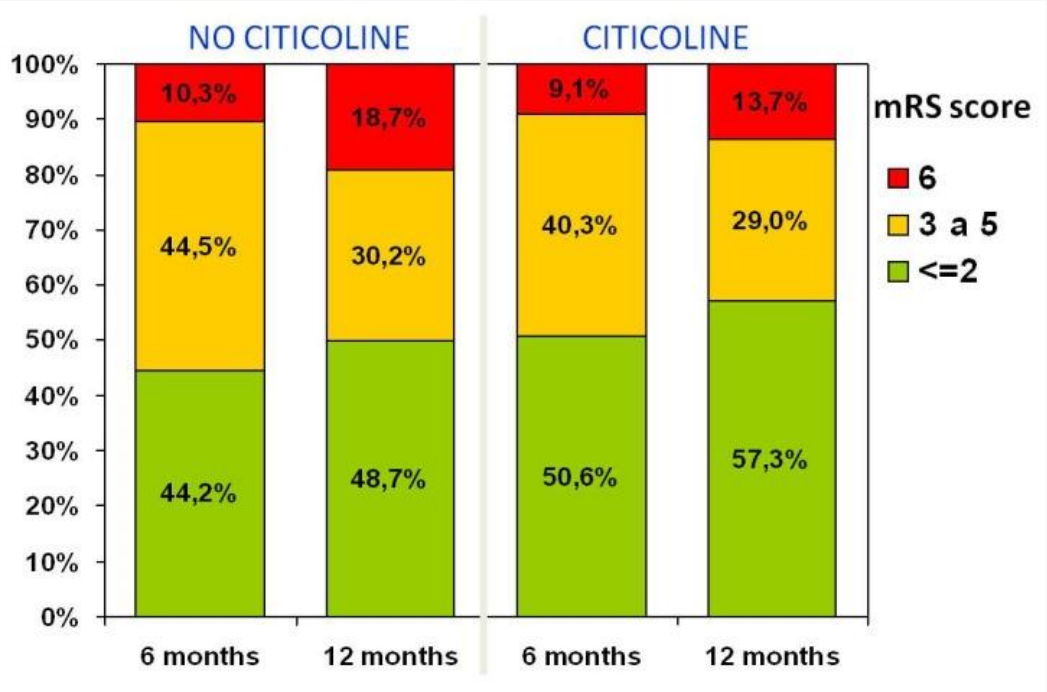


Figure 6. Functional status during follow-up: Notice the improvement in mRS scores (<2) at six and 12 months following stroke in the group treated with citicoline, compared with those untreated.

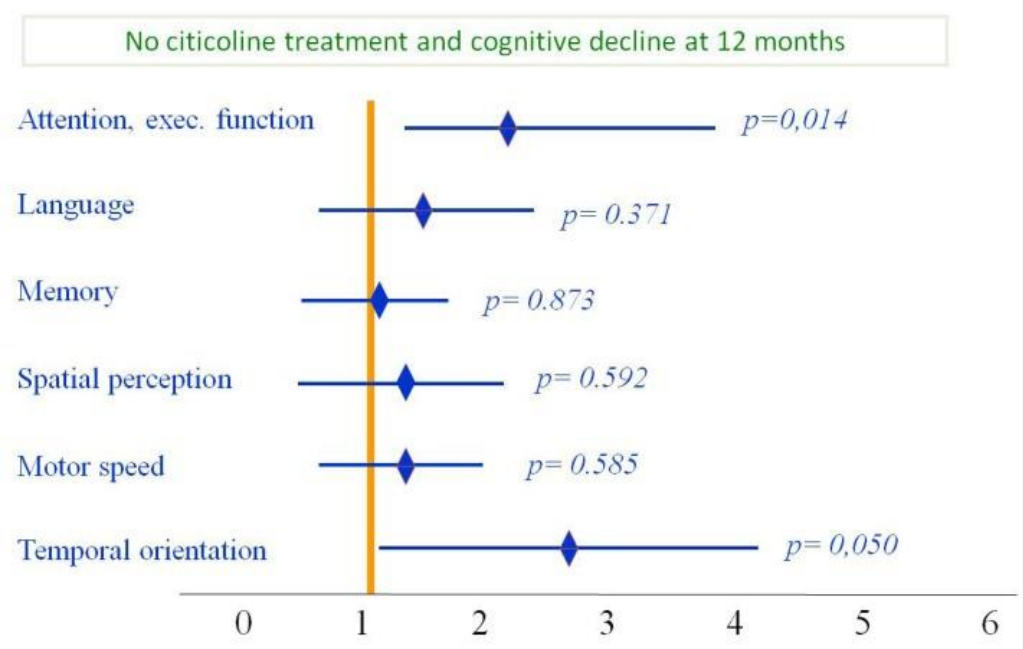


Figure 7. Functional status during six month follow-up: Subjects treated with citicoline had improvement on all cognitive domains; however, improvement was statistically significant only for attention/executive function and temporal orientation. Modified from Álvarez-Sabín *et al*.

References:

1. Alvarez-Sabín J, Román GC. The role of citicoline in neuroprotection and neurorepair in ischemic stroke. *Brain Sci*. 2013;3(3):1395-1414.
2. Secades JJ, Gareri P. Citicoline: pharmacological and clinical review, 2022 update. Citicolina: revisión farmacológica y clínica, actualización 2022. *Rev Neurol*. 2022;75(s05):S1-S89.
3. Alvarez-Sabín J, Román GC. The role of citicoline in neuroprotection and neurorepair in ischemic stroke. *Brain Sci*. 2013;3(3):1395-1414.

ABSTRACTS

Application of Citicoline in Neurological Disorders: A Systematic Review

Abstract

Citicoline is a chemical compound involved in the synthesis of cell membranes. It also has other, not yet explained functions. Research on the use of citicoline is conducted in neurology, ophthalmology, and psychiatry. Citicoline is widely available as a dietary supplement. It is often used to enhance cognitive functions. In our article, accessible databases were searched for articles regarding citicoline use in neurological diseases. This article has a systemic review form. After rejecting non-eligible reports, 47 remaining articles were reviewed. The review found that citicoline has been proven to be a useful compound in preventing dementia progression. It also enhances cognitive functions among healthy individuals and improves prognosis after stroke. In an animal model of nerve damage and neuropathy, citicoline stimulated regeneration and lessened pain. Among patients who underwent brain trauma, citicoline has an unclear clinical effect. Citicoline has a wide range of effects and could be an essential substance in the treatment of many neurological diseases. Its positive impact on learning and cognitive functions among the healthy population is also worth noting.

Reference: Jasielski P, Piędel F, Piwek M, Rocka A, Petit V, Rejdak K. Application of Citicoline in Neurological Disorders: A Systematic Review. *Nutrients*. 2020;12(10):3113.

Citicoline for Acute Ischemic Stroke: A Systematic Review and Formal Meta-analysis of Randomized, Double-Blind, and Placebo-Controlled Trials

Abstract

Background: Citicoline is a drug approved for the treatment of acute ischemic stroke. Although evidence of its efficacy has been reported, recently published results of a large placebo-controlled clinical trial did not show differences. This study aims to assess whether starting citicoline treatment within 14 days after stroke onset improves the outcome in patients with acute ischemic stroke, as compared with placebo.

Methods: A systematic search was performed to identify all published, unconfounded, randomized, double-blind, and placebo-controlled clinical trials of citicoline in acute ischemic stroke.

Results: Ten randomized clinical trials met our inclusion criteria. The administration of citicoline was associated with a significant higher rate of independence, independently of the method of evaluation used (odds ratio [OR] 1.56, 95% confidence interval [CI] = 1.12-2.16 under random effects; OR 1.20, 95% CI = 1.06-1.36 under fixed effects). After studying the cumulative meta-analysis, and with the results obtained with the subgroup of patients who were not treated with recombinant tissue plasminogen activator (rtPA) (OR 1.63, 95% CI = 1.18-2.24 under random effects; OR 1.42, 95% CI = 1.22-1.66 under fixed effects), our hypothesis of dilution of the effect of citicoline was confirmed. When we analyzed the effect of citicoline in patients who were not treated with rtPA and were receiving the highest dose of citicoline started in the first 24 hours after onset, based on more recent trials, there was no heterogeneity, and the size of the effect has an OR of 1.27 (95% CI = 1.05-1.53).

Conclusions: This systematic review supports some benefits of citicoline in the treatment of acute ischemic stroke. But, on top of the best treatment available (rtPA), citicoline offers a limited benefit.

Reference: Secades JJ, Alvarez-Sabín J, Castillo J, et al. Citicoline for Acute Ischemic Stroke: A Systematic Review and Formal Meta-analysis of Randomized, Double-Blind, and Placebo-Controlled Trials. *J Stroke Cerebrovasc Dis*. 2016;25(8):1984-1996.

Survey Form

1) In your clinical practice, what is the most common age group at which present with stroke?

a) 20-35 years
b) 36-50 years
c) 51-65 years
d) 66-80 years

2) In your clinical practice, how many patients per week present within 4.5 hours of symptom onset?

a) 1-4
b) 4-7
c) 7-10
d) >10

3) In your clinical practice what is the average door to needle time?

a) <30 mins
b) 30-60 mins
c) >60 mins

4) In your clinical practice, do you prefer giving citicoline after revascularisation therapy in patients of acute ischemic stroke?

a) Yes
b) No

5) In your clinical practice, what is the dose of intravenous citicoline you administer immediately after revascularization therapy for acute ischemic stroke?

a) 500 mg/ day
b) 1000 mg/ day
c) 2000 mg/ day
d) None of the above

6) What is the duration of orally administered citicoline post revascularization therapy in patients with acute ischemic stroke in your clinical practice?

a) 4 weeks
b) 6 weeks
c) 8 weeks
d) None of the above
e) I don't give oral citicoline post stroke

7) In which patients do you prefer to administer Citicoline after revascularisation therapy?

a) Mild stroke
b) Mild to Moderate stroke
c) Moderate to Severe stroke
d) Severe stroke

8) In your clinical practice, has administration of citicoline improved the functional outcomes at 90 days?

a) Yes
b) No

9) In your clinical practice, what is the percentage of patients with excellent functional outcome at 90 days in whom you have administered Citicoline after revascularisation therapy?

a) <20%
b) 21-40%
c) 41-60%
d) >60%

10) In your clinical practice, what is the percentage of patients with favourable functional outcome at 90 days in whom you have administered Citicoline after revascularisation therapy?

a) <20%
b) 21-40%
c) 41-60%
d) >60%

11) In your clinical practice, in which patient profile do you prefer to administer Citicoline?

a) Large Vessel Occlusion
b) Small Vessel Occlusion
c) Both

12) In your clinical practice, how will you rate the efficacy of citicoline in poststroke patients on below scale? (1 worse – 10 best)

a) 1
b) 2
c) 3
d) 4
e) 5
f) 6
g) 7
h) 8
i) 9
j) 10

13) In your clinical practice, on an average how long the patient takes oral citicoline after stroke?

a) < 50%
b) 50-70%
c) 71-90%
d) > 90%

14) In your clinical practice, what percentage of patients experienced side effect after citicoline therapy?

a) 5-15%
b) 15-25%
c) 25-35%
d) > 35%

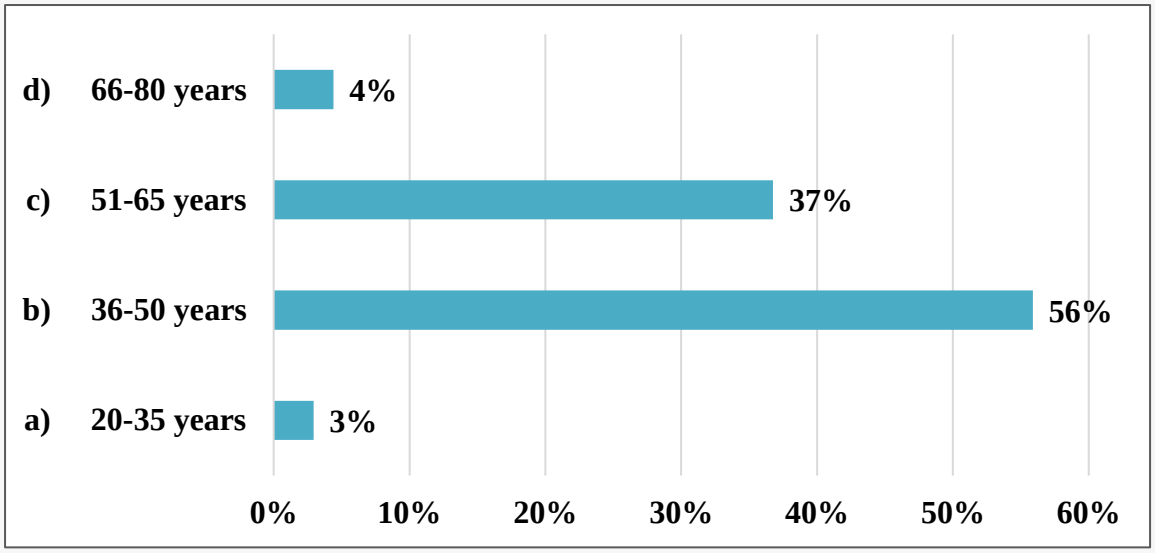
15) In your clinical practice, what is the most common side effect after citicoline therapy?

a) Diarrhoea
b) Leg oedema
c) Back pain
d) Headache
e) Well tolerated with no major adverse effects

Survey Findings

1) In your clinical practice, what is the most common age group at which present with stroke?

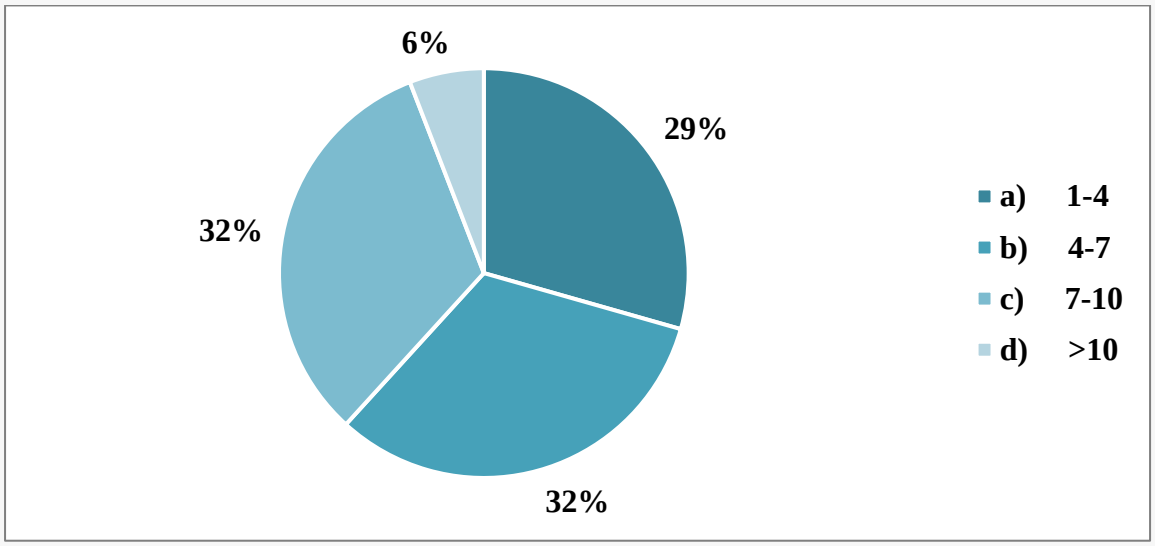
a) 20-35 years
b) 36-50 years
c) 51-65 years
d) 66-80 years



As per 56% of doctors, 36-50 years is the most common age group for patients which present with stroke.

2) In your clinical practice, how many patients per week present within 4.5 hours of symptom onset?

a) 1-4
b) 4-7
c) 7-10
d) >10



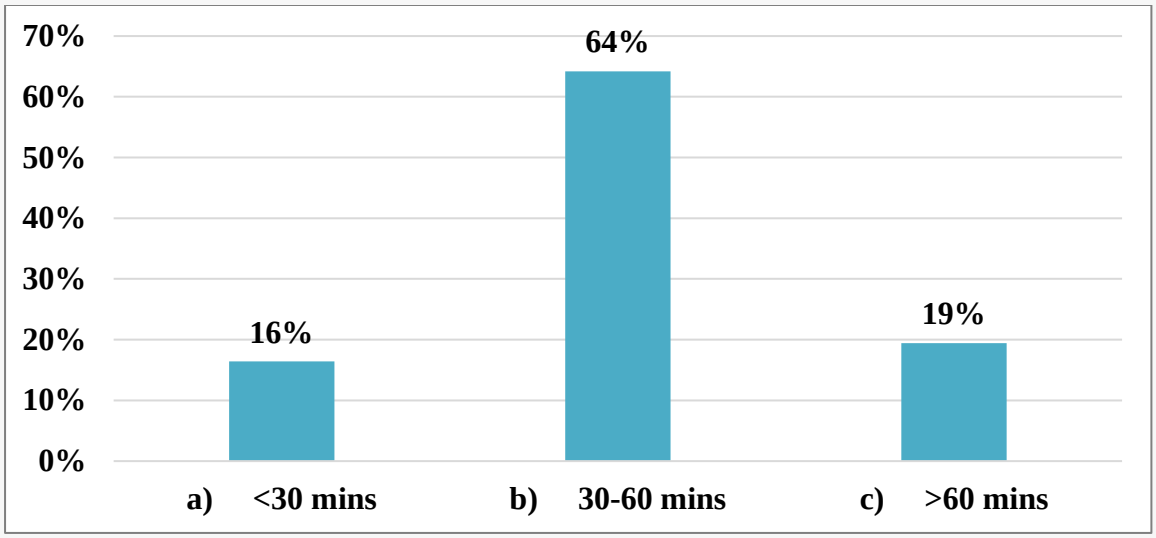
According to 32% of doctors, 4-7 patients per week present within 4.5 hours of symptom onset whereas as per another 32%, the number is 7-10.

3) In your clinical practice what is the average door to needle time?

a) <30 mins

b) 30-60 mins

c) >60 mins

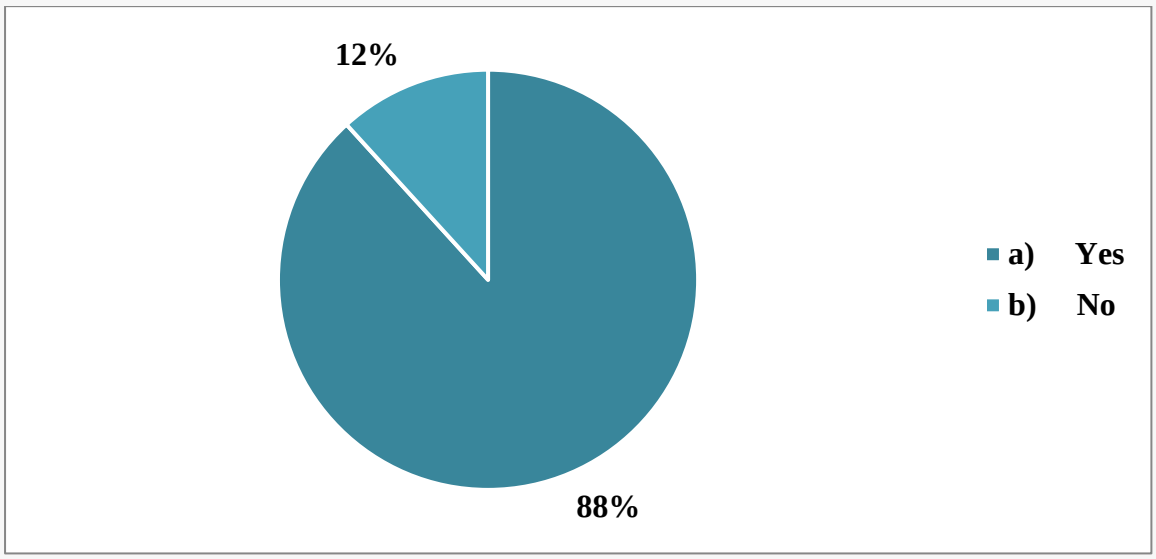


As per 64% of doctors, the average door to needle time is 30-60 mins.

4) In your clinical practice, do you prefer giving citicoline after revascularisation therapy in patients of acute ischemic stroke?

a) Yes

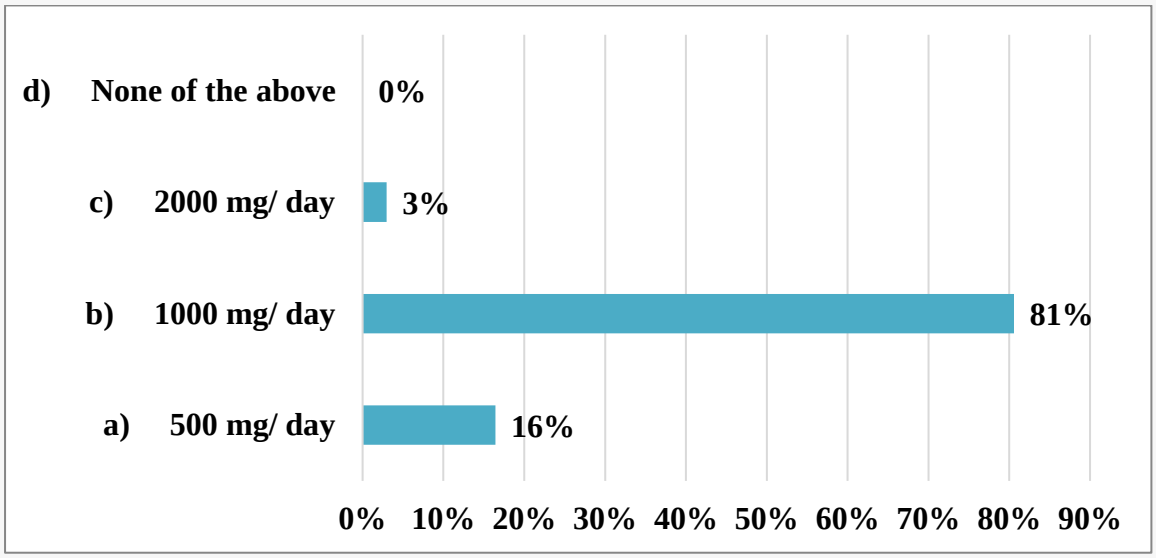
b) No



Majority of doctors, 88%, prefer giving citicoline after revascularisation therapy in patients of acute ischemic stroke.

5) In your clinical practice, what is the dose of intravenous citicoline you administer immediately after revascularization therapy for acute ischemic stroke?

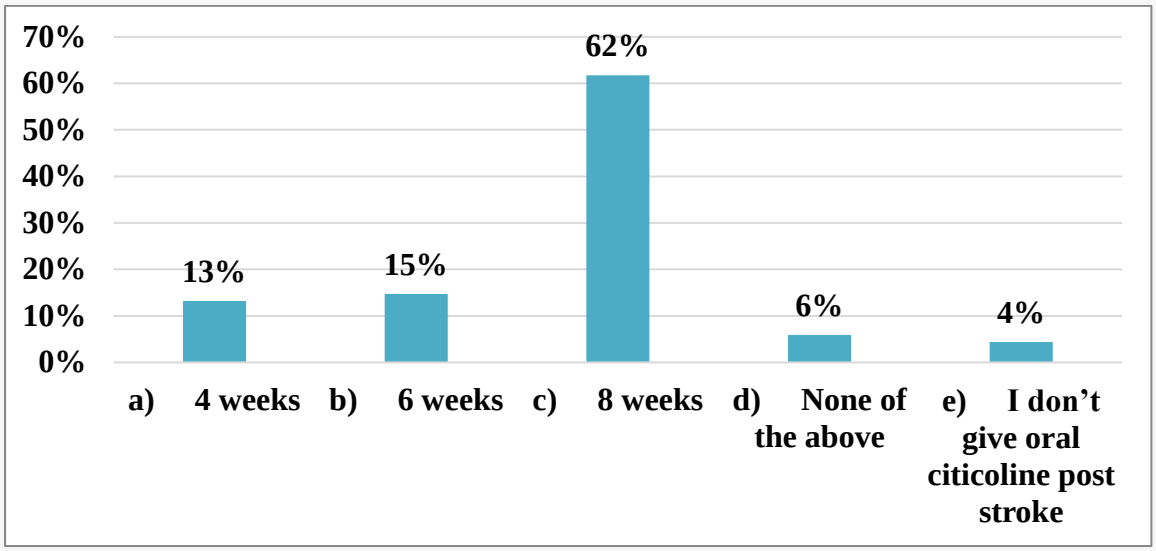
a) 500 mg/ day
b) 1000 mg/ day
c) 2000 mg/ day
d) None of the above



In the clinical practice of majority of doctors, 81%, the dose of intravenous citicoline they administer immediately after revascularization therapy for acute ischemic stroke is 1000mg/day.

6) What is the duration of orally administered citicoline post revascularization therapy in patients with acute ischemic stroke in your clinical practice?

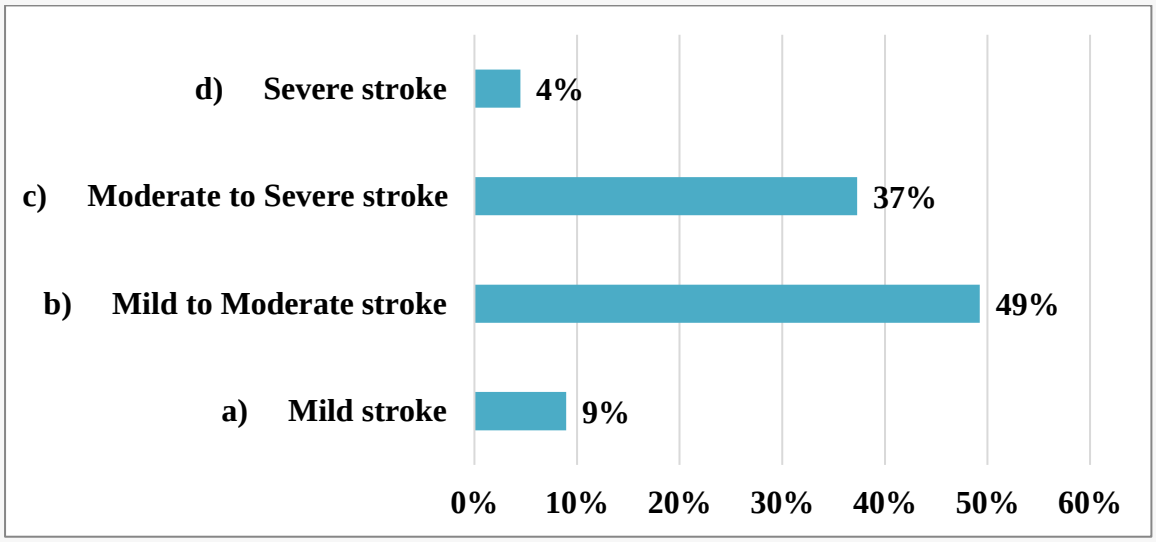
a) 4 weeks
b) 6 weeks
c) 8 weeks
d) None of the above
e) I don't give oral citicoline post stroke



According to 62% of doctors, the duration of orally administered citicoline post revascularization therapy in patients with acute ischemic stroke in their clinical practice is 8 weeks.

7) In which patients do you prefer to administer Citicoline after revascularisation therapy?

a) Mild stroke
b) Mild to Moderate stroke
c) Moderate to Severe stroke
d) Severe stroke

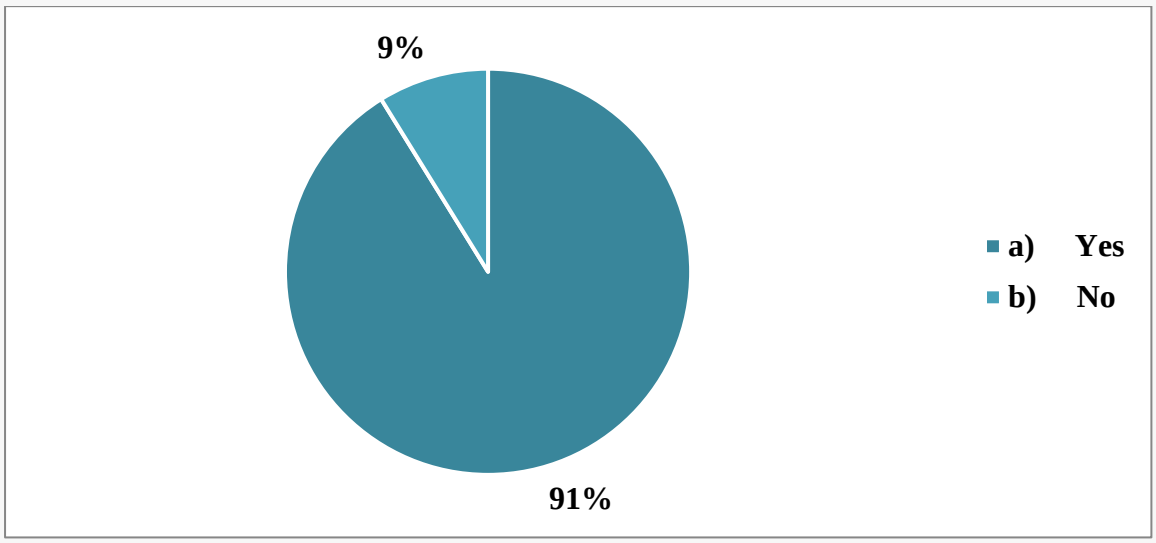


49% of doctors prefer to administer Citicoline after revascularisation therapy in patients having mild to moderate stroke.

8) In your clinical practice, has administration of citicoline improved the functional outcomes at 90 days?

a) Yes

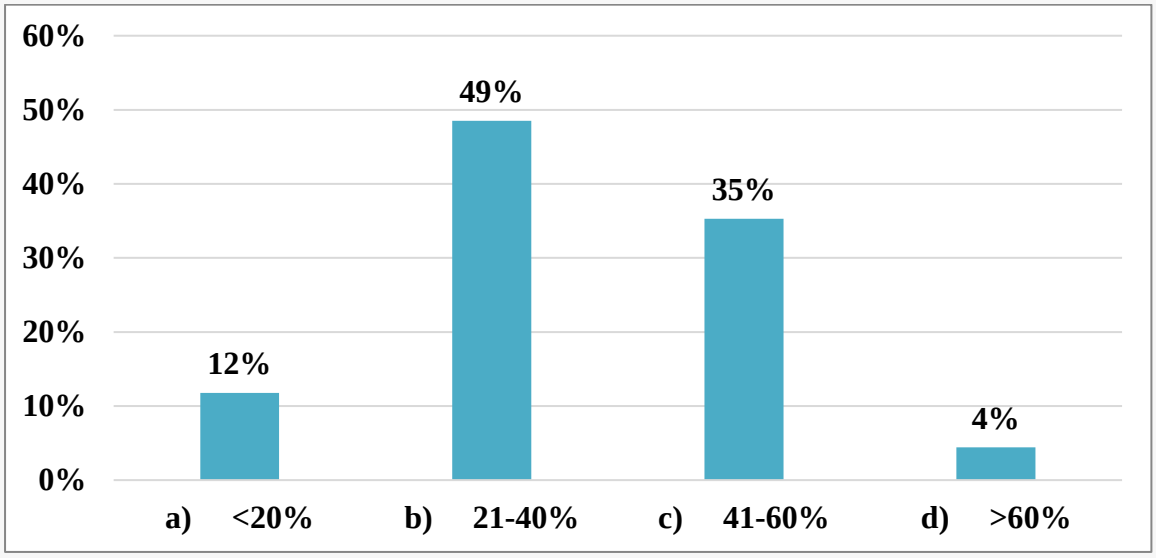
b) No



As per majority of doctors, 91%, the administration of citicoline has improved the functional outcomes at 90 days.

9) In your clinical practice, what is the percentage of patients with excellent functional outcome at 90 days in whom you have administered Citicoline after revascularisation therapy?

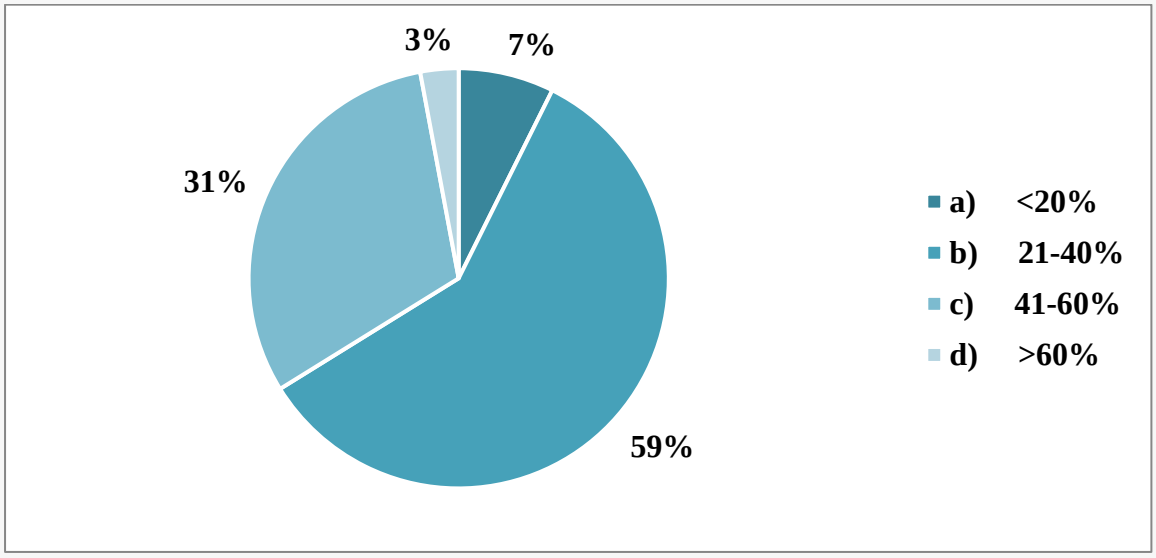
a) <20%
b) 21-40%
c) 41-60%
d) >60%



According to 49% of doctors, 21-40% of patients with excellent functional outcome at 90 days in whom they have administered Citicoline after revascularisation therapy.

10) In your clinical practice, what is the percentage of patients with favourable functional outcome at 90 days in whom you have administered Citicoline after revascularisation therapy?

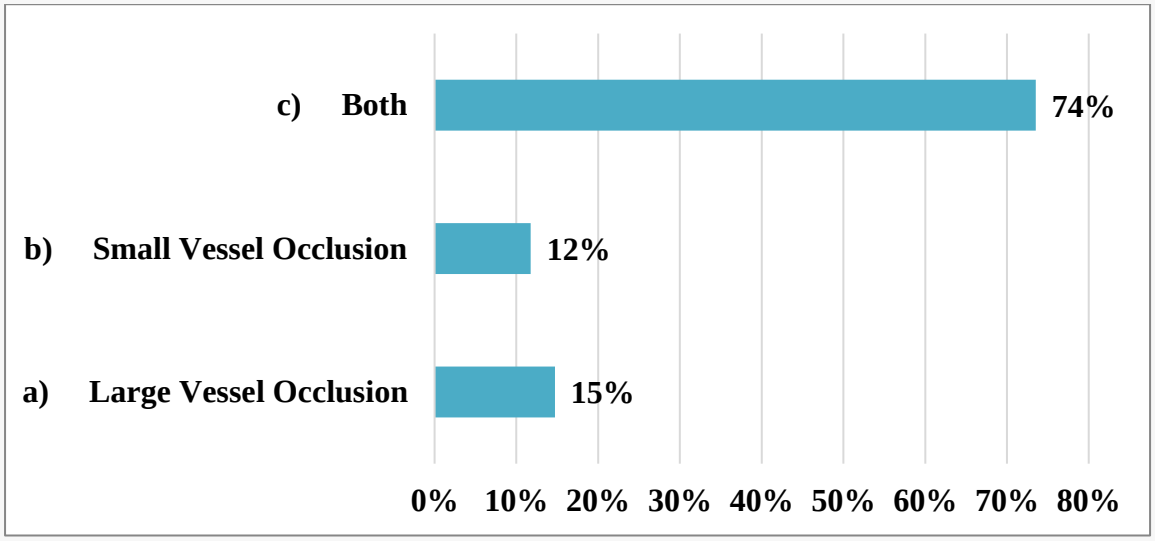
a) <20%
b) 21-40%
c) 41-60%
d) >60%



As per 59% of doctors, 21-40% of patients with favourable functional outcome at 90 days in whom they have administered Citicoline after revascularisation therapy.

11) In your clinical practice, in which patient profile do you prefer to administer Citicoline?

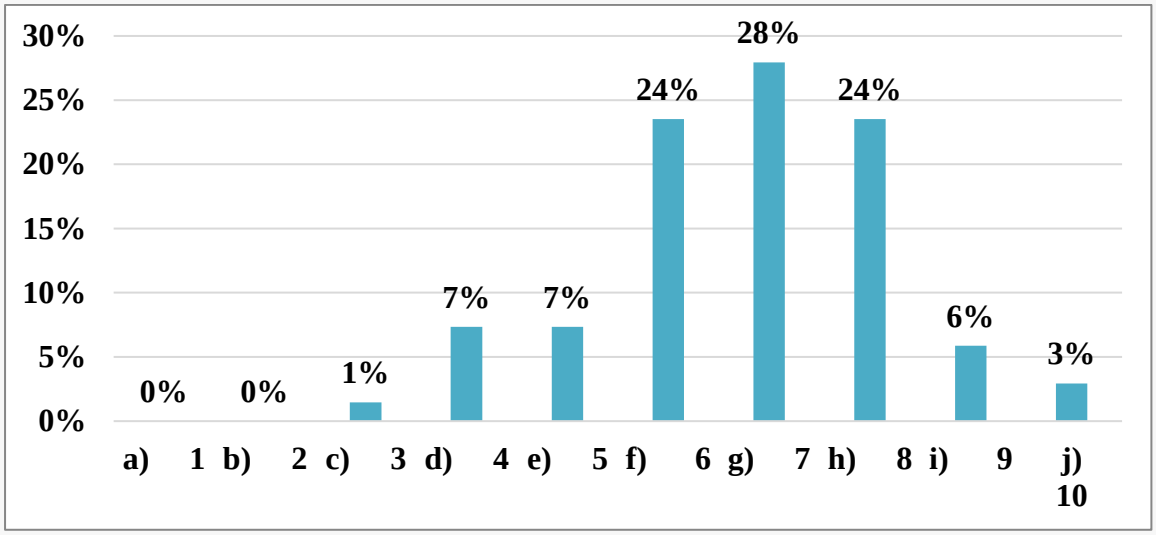
a) Large Vessel Occlusion
b) Small Vessel Occlusion
c) Both



Majority of doctors, 74%, prefer Large Vessel Occlusion and Small Vessel Occlusion for administering Citicoline.

12) In your clinical practice, how will you rate the efficacy of citicoline in poststroke patients on below scale? (1 worse – 10 best)

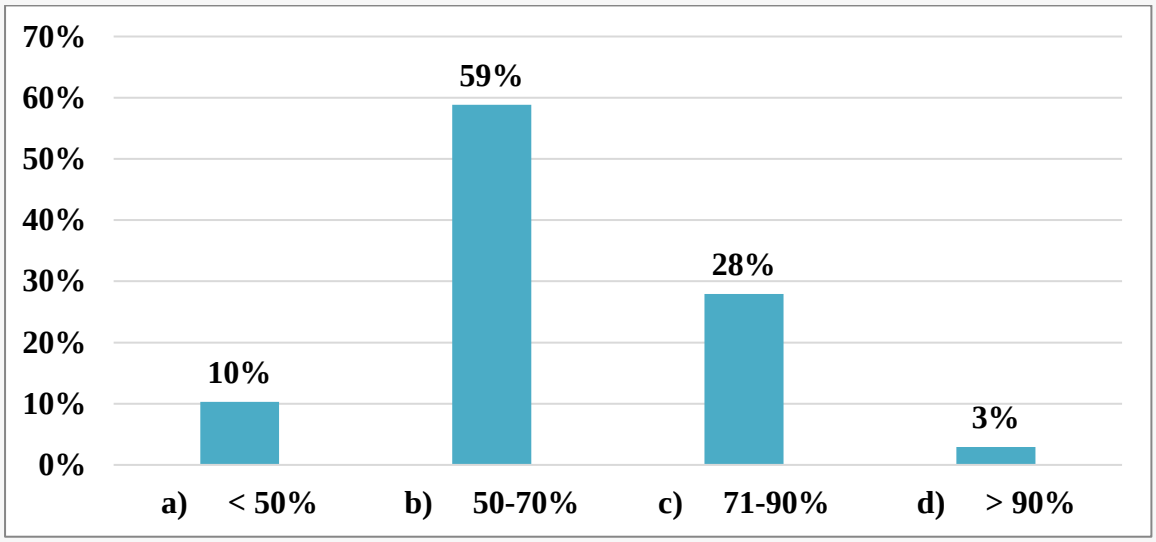
a) 1
b) 2
c) 3
d) 4
e) 5
f) 6
g) 7
h) 8
i) 9
j) 10



28% of doctors rate the efficacy of citicoline in poststroke patients as 7 on a scale of 1-10.

13) In your clinical practice, on an average how long the patient takes oral citicoline after stroke?

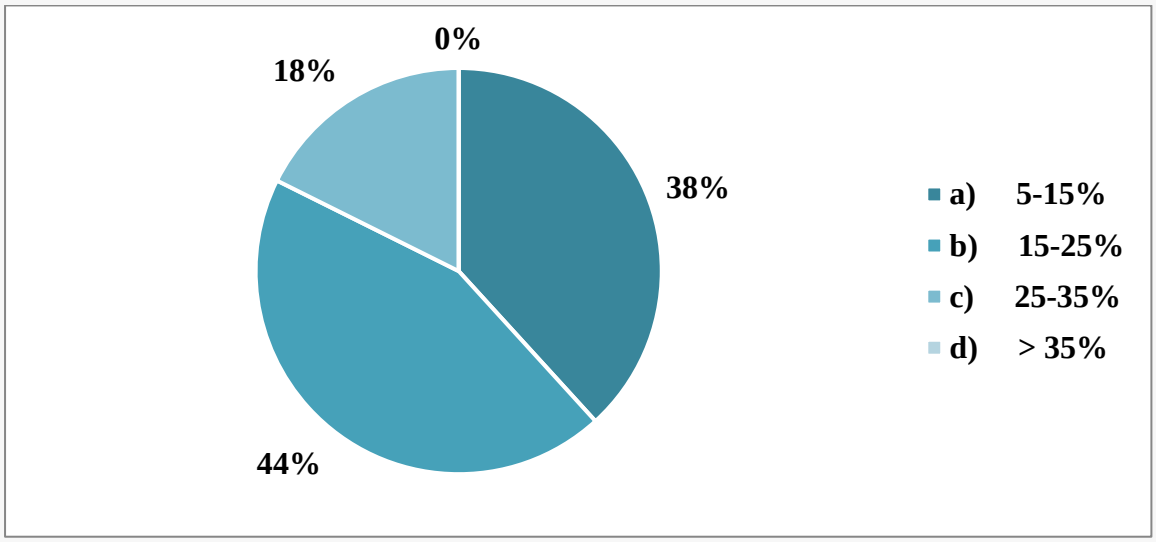
a) < 50%
b) 50-70%
c) 71-90%
d) 90%



In the clinical practice of 59% of doctors, on an average 50-70% patient takes oral citicoline after stroke.

14) In your clinical practice, what percentage of patients experienced side effect after citicoline therapy?

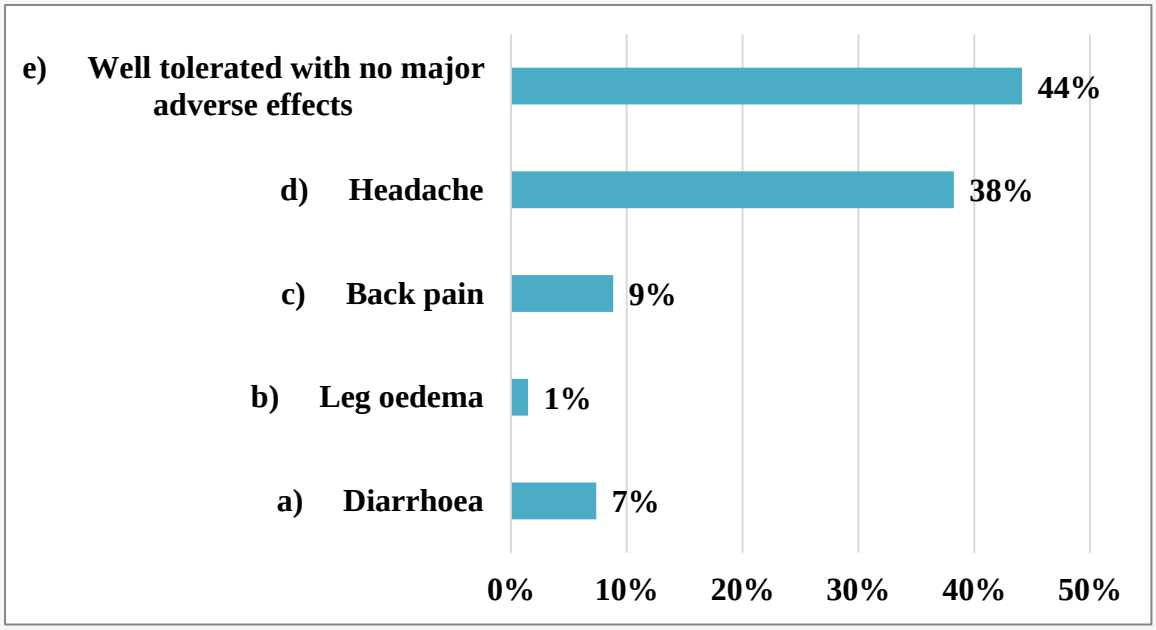
a) 5-15%
b) 15-25%
c) 25-35%
d) 35%



In the clinical practice of 44% of doctors, 15-25% of patients experience side effect after citicoline therapy.

15) In your clinical practice, what is the most common side effect after citicoline therapy?

a) Diarrhoea
b) Leg oedema
c) Back pain
d) Headache
e) Well tolerated with no major adverse effects



In the clinical experience of 44% of doctors, the citicoline therapy is well tolerated with no major adverse effects.

Summary

- As per 56% of doctors, 36-50 years is the most common age group for patients which present with stroke.
- According to 32% of doctors, 4-7 patients per week present within 4.5 hours of symptom onset whereas as per another 32%, the number is 7-10.
- As per 64% of doctors, the average door to needle time is 30-60 mins.
- Majority of doctors, 88%, prefer giving citicoline after revascularisation therapy in patients of acute ischemic stroke.
- In the clinical practice of majority of doctors, 81%, the dose of intravenous citicoline they administer immediately after revascularization therapy for acute ischemic stroke is 1000mg/day.
- According to 62% of doctors, the duration of orally administered citicoline post revascularization therapy in patients with acute ischemic stroke in their clinical practice is 8 weeks.
- 49% of doctors prefer to administer Citicoline after revascularisation therapy in patients having mild to moderate stroke.
- As per majority of doctors, 91%, the administration of citicoline has improved the functional outcomes at 90 days.
- According to 49% of doctors, 21-40% of patients with excellent functional outcome at 90 days in whom they have administered Citicoline after revascularisation therapy.
- As per 59% of doctors, 21-40% of patients with favourable functional outcome at 90 days in whom they have administered Citicoline after revascularisation therapy.
- Majority of doctors, 74%, prefer Large Vessel Occlusion and Small Vessel Occlusion for administering Citicoline.
- 28% of doctors rate the efficacy of citicoline in poststroke patients as 7 on a scale of 1-10.
- In the clinical practice of 59% of doctors, on an average 50-70% patient takes oral citicoline after stroke.

- In the clinical practice of 44% of doctors, 15-25% of patients experience side effect after citicoline therapy.
- In the clinical experience of 44% of doctors, the citicoline therapy is well tolerated with no major adverse effects.

Consultant Opinion

Patient Demographics:

Doctors commonly encounter patients aged between 36-50 years presenting with stroke. This suggests that stroke affects a relatively younger population, underscoring the importance of stroke prevention and management in adults of working age.

Presentation Within Time Window:

Doctors report a significant number of patients presenting within the crucial 4.5-hour time window from symptom onset. This timely presentation allows for the administration of time-sensitive interventions such as thrombolysis, potentially improving patient outcomes.

Door to Needle Time:

Efforts are made to ensure prompt treatment initiation, with an average door to needle time ranging from 30 to 60 minutes. This emphasizes the importance of streamlined processes in healthcare facilities to minimize treatment delays and optimize patient care.

Preference for Citicoline:

Citicoline is commonly favored by doctors for administration after revascularization therapy in patients with acute ischemic stroke. This indicates recognition of citicoline's potential neuroprotective and neurorestorative effects in the post-stroke period.

Dosage and Duration:

Doctors typically administer citicoline intravenously at a dose of 1000mg/day immediately after revascularization therapy, followed by oral administration for a duration of 8 weeks. This dosing regimen reflects an evidence-based approach aimed at maximizing the therapeutic benefits of citicoline.

Patient Selection:

Citicoline is often considered for patients with mild to moderate stroke severity after revascularization therapy. This tailored approach ensures appropriate treatment allocation based on individual patient characteristics and clinical needs.

Functional Outcomes:

Citicoline administration is associated with improved functional outcomes at 90 days post-stroke. This underscores its potential to enhance neurological recovery and reduce long-term disability in stroke survivors.

Preference for Occlusion Types:

Doctors prefer administering citicoline in patients with large vessel occlusion and small vessel occlusion strokes. This preference reflects an understanding of citicoline's potential efficacy in different stroke subtypes, guided by current evidence and clinical experience.

Efficacy Rating:

Citicoline is generally perceived as effective in post-stroke patients, with many doctors rating its efficacy favorably. This positive perception underscores confidence in citicoline as a valuable therapeutic option in the management of acute ischemic stroke.

Adherence and Side Effects:

While a significant proportion of patients adhere to oral citicoline therapy after stroke, a notable minority may experience side effects. Nevertheless, for a considerable number of patients, citicoline therapy is well tolerated, with minimal adverse effects reported by clinicians.

These findings highlight the multifaceted role of citicoline in the comprehensive management of acute ischemic stroke, encompassing neuroprotection, functional recovery, and long-term outcomes improvement.

NOTES

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