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Research Article

Pyrimidine Nucleoside-Mediate Prevention of Neurobehavioral Consequences Related to Cerebral Ischemia in Rat Models

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Abstracts

Introduction: Cerebral ischemia is one of the leading causes of mortality and long-term disability, such as anxiety, motor disbalance and memory loss. Endogenous compounds such as pyrimidine nucleosides have demonstrated neuroprotective properties in several neurological disorders. The objective of this investigation was to study the effects of uridine and cytidine combination on cerebral ischemia-related anxiety, motor disbalance and memory loss in a preclinical model.

Methods: Experiments were performed on inbred albino rats, which were remained intact or treated with either saline (control) or a combination of 5 mg/kg cytidine-5-mononucleic acid and 3 mg/kg uridine-phosphate salts. All animals were tested for elevated plus maze (EPM), passive avoidance (PAT) and rota-rod tests, which measure anxiety related behaviour and motor coordination, at baseline and on the 6th and 12th day after being subjected to middle cerebral artery occlusion (MCAO).

Results: Animals treated with nucleotides reported an improvement of about 28% and 73% compared to the control rats six and twelve days after MCAO, respectively, in the passive avoidance test ($p<0.05$ for both). Regarding elevated plus maze and rota-rod tests, statistically significant improvements were observed in all studied parameters in the nucleotide group compared to the control group.

Conclusion: Administration of pyrimidine nucleotides combination under ischemic conditions prevents deterioration of learning and memory processes, anxiety and motor coordination disbalance of rats. Therefore, these molecules might represent a novel therapeutic approach to treat cerebral ischemia-related neurobehavioral consequences.

Keywords: Cerebral ischemia; Pyrimidine nucleotides; Anxiety; Memory disorders; Discoordination

Abbreviations: CBF: Cerebral Blood Flow; CMP: Cytidine Monophosphate; CTP: Cytidine Triphosphate; EPM: Elevated Plus Maze; GABA: Gamma-Amino Butyric Acid; MCAO: Middle Cerebral Artery Occlusion; PAT: Passive Avoidance Test; PC: Phosphatidyl Choline; SEM: Standard Error Of Mean; UTP: Uridine Triphosphate

Introduction

Cerebral ischemia is one of the worldwide leading causes of morbidity and mortality. Neurobehavioral consequences linked to cerebral ischemia include a wide range of disorders such as anxiety, motor disbalance and memory loss [1]. Many drugs have been

proposed to interrupt the ischemic cascade, whose neuroprotective efficiency is tested through their ability to prevent mood changes in animals with cerebral ischemia. Two pyrimidine nucleosides, cytidine and uridine, have been proposed among these medications. Both nucleosides play an important role during the regulation of brain functions [2], since it has been described that concentration of these compounds are decreased under ischemic conditions [3,4]. Cytidine monophosphate (CMP) mediates the synthesis of the complex lipids that form part of the neuronal membrane, particularly sphingomyelin, which is the parent substance of the myelin sheath as well as a precursor of DNA and RNA nucleic acids [1,5-7]. On the other hand, uridine (as a precursor metabolite) supports the synthesis of different molecules involved in diverse neural processes. Therefore, uridine administration has been proposed to improve memory function and affected neuronal plasticity. These effects seem to be mediated by the promotion of neuronal membrane formation and through interactions with specific uridine-nucleotide receptors (brain P2Y2 receptors) that control neuronal differentiation [8]. Furthermore, uridine triphosphate (UTP) may be converted into cytidine triphosphate (CTP), which is a key intermediate nucleotide in the Kennedy cycle, which is involved in the generation of phosphatidyl choline (PC) during the synthesis of neuronal membranes. This molecule acts as a coenzyme in the synthesis of glycolipids of the neuronal structures and the myelin sheath, complementing the action of CMP. In addition, it is also considered a cell energy provider2,3. Moreover, it has been described that uridine stimulates the increase of gamma-amino butyric acid (GABA) levels in brain [9], a molecule important in the prevention of GABA-glutamate ischemic disbalance [10].

Our research group has previously described that the combination of cytidine-5-mononucleic acid and uridine-phosphate salts improved cerebral blood flow (CBF) [11], which prevents the morphological changes in the brain tissue of rats with local cerebral ischemia caused by the middle cerebral artery occlusion (MCAO) [12]. Considering the results regarding the nucleoside synthesis disturbances observed during ischemic strokes, the objective of this study was to describe the effect of these compounds on anxiety, memory disorders, learning ability and lack of motor coordination caused by cerebral ischemia.

Materials and Methods

Experiments were performed on inbred albino rats weighting 160-180 g (between 9 a.m. and 2 p.m.). The rats were housed in standard laboratory cages (no more than five rats per each) with free access to both water and food in a dedicated animal vivarium. Environmental conditions were set at a 22-25°C temperature and a 40-70% humidity, under a 12-hour day/night cycle. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals Experiments. The study was approved by the Ethics Committee of Yerevan State Medical University (Yerevan, Armenia).

Surgical Procedure of Left Middle Cerebral Artery Occlusion (MCAO)

Rats were intraperitoneally anesthetized with 400 mg/kg chloral hydrate dissolved in 0.9% NaCl. The surgical procedures were performed following the protocol of Tamura et al [13] modified by Topchyan [14]. All rats were submitted to the elevated plus maze (EPM) [15,16], passive avoidance (PAT) [15] and rota-rod [17] tests both before MCAO and six and 12 days afterwards. Therefore, animals were divided into three groups: an intact group of animals consisting in 40 rats which were tested before MCAO (I group); a control group consisting in 14 rats injected once daily with 0.9% NaCl which were tested on the 6th and 12th day after MCAO (II group); and a treated group of animals consisting in 26 rats injected once daily with a combination of 5 mg/kg cytidine-5-mononucleic acid and 3 mg/kg uridine-phosphate salts which were tested on the 6th and 12th day after MCAO (III group).

Evaluation of Learning and Memory Processes

Both learning and memory processes of studied animals were evaluated through the passive avoidance test. Animals were placed in a platform, which is directly connected to an electrode device, located at a height of 50 cm inside a dark chamber. When the platform was lit the door was opened. If the animals stepped into the dark section of the chamber with their four paws, the door was closed, and a 3-second 0.3-0.6 mA foot shock was applied as a pain stimulus. After this step, the rats were placed in their respective home cage. The conditional reflex reaction of passive avoidance test was performed 24 hours later. The animals were placed back into the lit platform, recording for three minutes the latent time until it entered the dark chamber, with no electrical shocks being applied this time. Animals were considered educated if they stayed on the lit platform during all this period. Only rats with a formed reflex were included in subsequent experiments.

Measurement of Anxiety-Related Behaviour

Evaluation of anxiety-like behaviour was assessed in the elevated plus maze (EPM) test. The plus-maze was made of black wood, and it consisted of a 10x10 cm central platform, two opposite 50x10 cm arms and enclosed 50x10x40 cm arms, which were elevated 50 cm above the ground floor. The EPM was performed in a blight lighted room, with the placement of the plus-maze and lightning conditions remaining identical for each trail. Animals were placed in the central apparatus facing the open arms. During five minutes several behavioral parameters such as total time spent on the open arms, total time spent on the closed arms, number of entries into the open arms, total number of crosses and canter square time were recorded. After every trial the maze was cleaned with 70% ethanol.

Evaluation of Motor Coordination

Maximum running capacity was measured through a motor coordination test, the rota-rod treadmill for rats (accelerating model 7750, Ugo Basile, Varese, Italy). The rats were placed on the rotating drum with an acceleration rotor mode (10 speed points ranging from 4 to 40 rpm during five minutes). Performance time was scored as the time in second until the animals fell from the rotating shaft. Rats were trained for 2-3 trails during 1-2 minutes per day for 2-3 hours before MCAO, which represented the baseline values. During the experiments, the animals were hold by their tail and placed on the rotating drum in the direction opposite to the rotation of the rod, with the rotation speed set at 16 rpm, if

necessary. Two or three measurements were performed for each animal.

Data Analysis

All data were represented as mean and standard error of mean (SEM). Comparisons between groups were made through the student's paired t-test or one-way ANOVA test followed by post hoc comparisons by Dunnet, where applicable. Statistical significance was established at $p<0.05$. All analyses were performed using SPSS software package, version 20.0.

Results and Discussion

Results

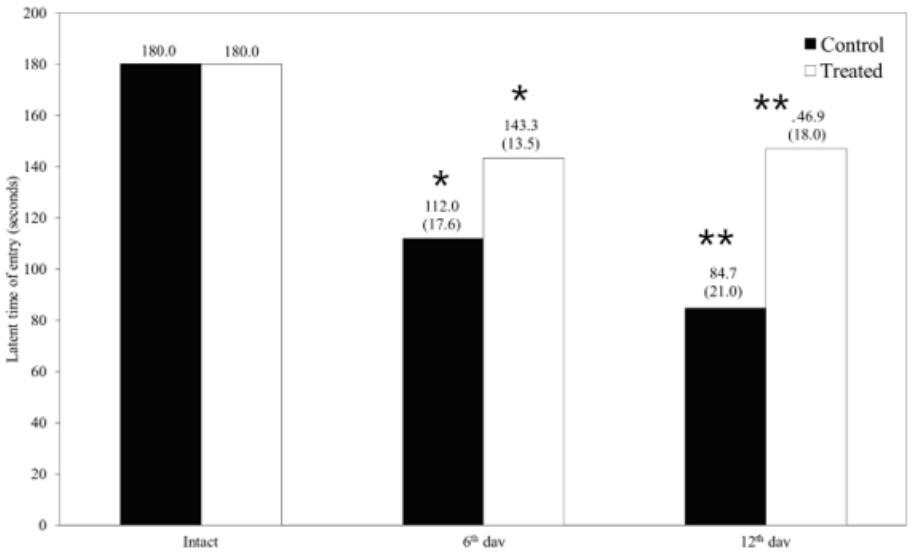

Figure 1: Changes of latent time of animals' entry in the Passive Avoidance Test after i/p injection of cytidine-5-mononucleic acid (5 mg/kg) and uridine-phosphate salts (3 mg/kg) combination on the 6th and 12th days after MCAO. Each value represents the mean ± SEM. Significant differences: *p<0.05 compared with the intact group, **p<0.05 compared with the control group.

In the control sample, a significant reduction of the latent time of animal entry into the dark compartment was observed, which went from 180.0 to 112.0±17.6 and 84.8±21.0 seconds ($p<0.05$ for both frames) six and 12 days after MCAO was performed. In comparison, intraperitoneal administration of cytidine and uridine nucleosides combination, provoked an improvement of these values, since from 180 seconds at baseline, the latent time diminished to 143.3±13.5 and 146.9±18.0 seconds on the 6th and 12th day post-MCAO ($p<0.05$ for both). These values represented an improvement of about 28% and 73% compared to the control animal models (Figure 1).

Regarding EPM test results, an anxiety development due to MCAO was observed in the control group. After six days a decrease in all the parameters was observed compared with the intact group (90.0% in the time spent on the open arms, 66.5% in the center square time, 82.6% in total number of entrances and 63% in the percentage of open arms entrances). In the case of the treatment group, an improvement in all EPM parameters was reported in comparison to the control sample at the same time frame. While the time spent on open arms improved by 1090.2% (38.9±14.6 vs 3.3±2.1 seconds, $p<0.05$), this increase was of 270.4% for centre square time (78.5±22.8 vs 21.2±17.9 seconds, $p<0.05$), 40.8% for the total number of entrances (1.7±0.2 vs 1.2±0.2, $p>0.05$), and 123.1% for the percentage of open arms entrances (39.5±6.9 vs 17.7±8.3, $p<0.05$). This difference between the control and treated animals was enhanced 12 days after MCAO in all the studied parameters, with the values obtained in the latter group remaining above the former (Table 1).

Table 1: Changes in the EPM test parameters after intraperitoneal injection of 5 mg/kg cytidine-5-mononucleic acid and 3 mg/kg uridine-phosphate salts combination on the 6th and 12th day post-MCAO.

EPM parameters	Intact group	After 6 days of MCAO		After 12 days of MCAO	
		Control	Treated	Control	Treated
OT	32.1±6.5	3.3±2.1*	38.9±14.6**	1.1±0.7*	63.7±26.4**
CT	63.7±14.2	21.2±17.9*	78.5±22.8**	3.8±1.9*	75.0±28.1**

TNE	6.9±1.1	1.2±0.2*	1.7±0.2	1.4±0.2*	1.9±0.2
POE (%)	47.6±5.4	17.7±8.3*	39.5±6.9**	20.0±11.1*	51.7±9.9**

Each value represents the mean ± SEM. Significant differences: *p<0.05 compared with the intact group, **p<0.05 compared with the control group. OT, time in seconds spent on the open arms; CT, centre square time in seconds; TNE, total number of entrances; POE (%), percentage of open arms entrances. SEM: standard error of mean; MCAO: middle cerebral artery occlusion.

Regarding motor coordination, in the control group the fall down time changed from 131.4±19.8 to 38.6±5.8 seconds (a 70.5% reduction) and from 131.4±19.8 to 19.6±4.7 seconds (a decrease of about 85%) six and twelve days after MCAO compared to the intact group (p<0.05 for both). On the other hand, in the nucleoside treatment sample, this decrease was lower on the 6th day (from 131.4±19.8 to 63.9±10.8 seconds, p<0.05), with rats on the 12th day achieving better values compared to the intact animals (from 131.4±19.8 to 149.8±42.3 seconds, p<0.05; (Figure 2).

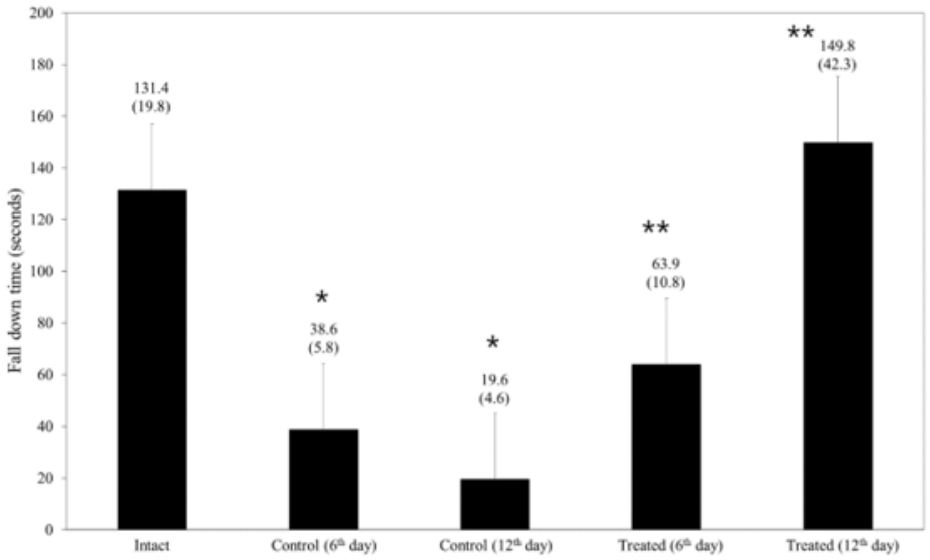

Figure 2: Changes of fall down time of animals in the Rota-rod test after i/p injection of cytidine-5-mononucleic acid (5 mg/kg) and uridine-phosphate salts (3 mg/kg) combination on the 6th and 12th days after MCAO. Each value represents the mean ± SEM. Significant differences: *p<0.05 compared with the intact group, **p<0.05 compared with the control group.

Discussion

Pyrimidine nucleosides such as cytidine (as cytidine triphosphate or CTP) and uridine, which is first converted to uridine triphosphate (UTP) and then to CTP, contribute to the biosynthesis of phosphatidylcholine and phosphatidylethanolamine in the brain via the Kenney pathway. Due to their central role in this pathway, the therapeutic uses of cytidine or uridine have been evaluated in neurological disorders such as acute or chronic ischemic stroke, traumatic brain injury and cognitive impairment [18]. Regarding uridine, a study in rat models with cerebral ischemia reperfusion injury showed that a dose of 90 µg/kg uridine 5'-triphosphate provided a statistically significant protective effect against cerebral ischemia reperfusion injury compared to saline (15.9% vs 30.5%, p<0.01) [19]. In neonatal rat models with hyperoxia-induced brain damage, a five-day uridine treatment decreased the number of apoptotic TUNNEL (+) cells in both CA1 (from 22.9 ± 0.9 to 15.3 ± 1.0 cells, p<0.001) and CA3 (from 16.6 ± 0.9 to 12.5 ± 1.3 cells, p<0.05) regions of the hippocampi compared to saline. This reduced apoptotic cell death due to uridine enhanced learning and memory performances after 40 days [20]. In this line, more recently, Al et al [21] described that antioxidative properties of uridine might mediate the neuroprotective effects of this molecule in these rat models. De Bruin et al [22] showed that administration of uridine and choline as a dietary supplementation led to improved selective attention and spatial learning in rat models used for testing cognitive impairment. Finally, supplementation of uridine monophosphate daily through a four-week period to the diet of gerbils increased the levels of major phospholipids such as of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol by 13%, 29% and 48%, respectively [23].

While there are some studies describing endogenous administration of uridine as a dietary supplementation for several neurological disorders, in the case of cytidine its supplementation as a nucleoside has not been studied. However, results from trials studying the protective effect of CDP-choline or citicoline, an endogenous intermediate produced during phosphatidylcholine biosynthesis in the Kennedy pathway, are available. Aslan et al [24] reported that topical administration of both this compound and

the combination of its metabolites, cytidine, and choline, improved functional recovery and promoted regeneration of surgically repaired injured sciatic nerves in rat models. The median nerve adherence and separability scores were 3.0 (range 2.0-3.0) for control, 1.5 (range 1.0-2.0) for CDP-choline, 1.5 (range 1.0-2.0) for cytidine, 2.0 (range 2.0-3.0) for choline and 1.5 (range 1.0-2.0) for cytidine+choline groups 12 weeks after sciatic nerve repair in those rats in which sciatic nerve was repaired on the first day. In the case of orally administered citicoline, it improved impaired memory-related behaviour and delayed neuronal cell death in mice with transient brain ischemia that underwent bilateral common carotid artery occlusion [25]. More recently, in toxic cuprizone-induced de- and remyelination mice CDP-choline improved the expression of myelin proteolipid protein (p=0.003) [26]. The neuroprotective effects of CDP-choline have been linked to an improved phospholipid synthesis, glucose metabolism and cholinergic function, as well as a reduced activation of both phospholipase A2 and apoptotic pathways [27-31].

Conclusion

The results obtained in this study describe the potential viability of these nucleotides as effective agents for ischemic brain protection. Here we demonstrate that the combination of endogenous molecules cytidine-5-mononucleic acid and uridine-phosphate salts remove the neurobehavioral consequences related to cerebral ischemia, preventing deterioration of both learning and memory processes, anxiety and motor coordination disbalance of rat animal models that underwent MCAO. These results are consistent with those reported in previous studies in which this combination of nucleotides improved cerebral blood flow and prevented morphological changes on brain tissue in local cerebral ischemic conditions. Altogether, these results suggest that these compounds might become a promising base to design and develop new neuroprotective agents aimed at tacking neurobehavioral consequences linked to cerebral ischemia.

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Conflicts of Interest

No conflict of interest.

References

1. Fornai F, Busceti CL, Ferrucci M, Lazzeri G, Ruggieri S (2002) Is there a role for uridine and pyrimidine nucleosides in the treatment of vascular dementia? Funct Neurol 17(2): 93-9.
2. Dobolyi A, Juhasz G, Kovacs Z, Kardos J (2011) Uridine function in the central nervous system. Curr Top Med Chem 11(8): 1058-67.
3. Baumel BS, Doraiswamy PM, Sabbagh M, Wurtman R (2021) Potential neuroregenerative and neuroprotective effects of uridine/Choline-Enriched multinutrient dietary intervention for mild cognitive impairment: a narrative review. Neurol Ther 10(1): 43-60.
4. Alvarez Sabin J, Roman GC (2013) The role of citicoline in neuroprotection and neurorepair in ischemic stroke. Brain Sci 3(3): 1395-414.
5. Gella A, Martianez T, Lamarca A, Gutierrez C (2011) A nucleotide-based drug protects against glutamate- and MPP+- induced neurotoxicity. Neuroscience and Medicine 2(2): 154-60.
6. Blunsom NJ, Cockcroft S (2020) CDP-Diacylglycerol Synthases (CDS): Gateway to phosphatidylinositol and cardiolipin synthesis. Front Cell Dev Biol 8: 63.
7. Manhaes M, Cesar M, Justo R, Geller M, Suchmacher M, et al. (2017) The role of nucleotides in glial cells during peripheral nerve trauma and compressive disorders. In: Mauricio, A. C., editor. Peripheral Nerve Regeneration - From Surgery to New Therapeutic Approaches Including Biomaterials and Cell-Based Therapies Development [Internet]. London: IntechOpen.
8. Von Kugelgen I, Hoffmann K (2016) Pharmacology and structure of P2Y receptors. Neuropharmacology 104: 50-61.
9. (2010) Sponsor: Mclean Hospital Collaborator: National Institute of Mental Health (NIMH) Information provided by: Mclean Hospital, Effect of uridine on GABA and high energy phosphate levels in healthy volunteers (Uridine-GABA), USA.
10. Belov Kirdajova D, Kriska J, Tureckova J, Anderova M (2020) Ischemia-triggered glutamate excitotoxicity from the perspective of glial cells. Front Cell Neurosci 14: 51.
11. Khachaturyan M, Balasanyan M (2012) Influence of pyrimidine nucleosides on cerebral blood flow, «YSMU annual conference report-2012, Collection of scientific articles». Erevan 86-92.
12. Khachatryan MS, Topchian HV, Gaspary HV, Balsani MG, Yerevan EL, et al. (2019) The effects of pirimidine nucleotides on the structural changes of rats' brain tissue under local ischemia. Journal Medical Science of Armenia 59(1): 45-54.
13. Tamura A, Graham DI, McCulloch J, Teasdale GM (1981) Focal cerebral ischaemia in the rat: 1. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. J Cereb Blood Flow Metab 1(1): 53-60.
14. Topchian AV, Mirzoian RS, Balasanian MG (1996) Local cerebral ischemia in rats induced by ligation of the middle cerebral artery. Eksp Klin Farmakol 59(5): 62-4.
15. Divya V, Pal SN, Pillai KK (2000) Effect of locomotor activity on the passive avoidance test for the evaluation of cognitive function. Indian Journal of Pharmacology 32: 242-5.
16. Leo LM, Pamplona FA (2014) Elevated Plus Maze Test to Assess Anxiety-like Behavior in the Mouse. Bioprotocol 4(16): e1211.
17. Deacon RM (2013) Measuring motor coordination in mice. J Vis Exp (75): e2609.
18. Cansev M (2006) Uridine and cytidine in the brain: their transport and utilization. Brain Res Rev 52(2): 389-97.
19. Tian ML, Zou Z, Yuan HB, Wang CC, Zhu QF, et al. (2009) Uridine 5'-triphosphate (UTP) protects against cerebral ischemia reperfusion injury in rats. Neurosci Lett 465(1): 55-60.
20. Goren B, Cakir A, Sevinc C, Serter Kocoglu S, Ocalan B, et al. (2017) Uridine treatment protects against neonatal brain damage and long-term cognitive deficits caused by hyperoxia. Brain Res 1676: 57-68.
21. Al N, Cakir A, Koc C, Cansev M, Alkan T (2020) Antioxidative effects of uridine in a neonatal rat model of hyperoxic brain injury. Turk J Med Sci 50(8): 2059-2066.
22. De Bruin NM, Kiliaan AJ, De Wilde MC, Broersen LM (2003) Combined uridine and choline administration improves cognitive deficits in spontaneously hypertensive rats. Neurobiol Learn Mem 80(1): 63-79.

23. Wurtman RJ, Ulus IH, Cansev M, Watkins CJ, Wang L, et al. (2006) Synaptic proteins and phospholipids are increased in gerbil brain by administering uridine plus docosahexaenoic acid orally. Brain Res 1088(1): 83-92.

24. Aslan E, Kocaeli H, Bekar A, Tolunay S, Ulus IH (2011) CDP-choline and its endogenous metabolites, cytidine, and choline, promote the nerve regeneration and improve the functional recovery of injured rat sciatic nerves. Neurol Res 33(7): 766-73.

25. Nakazaki E, Yabuki Y, Izumi H, Shinoda Y, Watanabe F, et al. (2019) Combined citicoline and docosahexaenoic acid treatment improves cognitive dysfunction following transient brain ischemia. J Pharmacol Sci 139(4): 319-324.

26. Gudi V, Schafer N, Gingele S, Stangel M, Skripuletz T (2021) Regenerative Effects of CDP-Choline: A Dose-Dependent Study in the Toxic Cuprizone Model of De- and Remyelination. Pharmaceuticals (Basel) 14(11): 1156.

27. Kakihana M, Fukuda N, Suno M, Nagaoka A (1988) Effects of CDP-choline on neurologic deficits and cerebral glucose metabolism in a rat model of cerebral ischemia. Stroke 19(2): 217-22.

28. Lopez Coviella I, Agut J, Savci V, Ortiz JA, Wurtman RJ (1995) Evidence that 5'-cytidinediphosphocholine can affect brain phospholipid composition by increasing choline and cytidine plasma levels. J Neurochem 65(2): 889-94.

29. Dixon CE, Ma X, Marion DW (1997) Effects of CDP-choline treatment on neurobehavioral deficits after TBI and on hippocampal and neocortical acetylcholine release. J Neurotrauma 14(3): 161-9.

30. Krupinski J, Ferrer I, Barrachina M, Secades JJ, Mercadal J, et al. (2002) CDP-choline reduces pro-caspase and cleaved caspase-3 expression, nuclear DNA fragmentation, and specific PARP-cleaved products of caspase activation following middle cerebral artery occlusion in the rat. Neuropharmacology 42(6): 846-54.

31. Adibhatla RM, Hatcher JF (2003) Citicoline decreases phospholipase A2 stimulation and hydroxyl radical generation in transient cerebral ischemia. J Neurosci Res 73(3): 308-15.