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PROTECTIVE EFFECT OF CITICOLINE IN ACUTE REVERSIBLE CEREBRAL ISCHEMIA IN RATS

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Resume. *Revascularization intervention for ischemic stroke has significant time and technological limitations. Therefore, the search for neuroprotective agents remains relevant.*

The aim of the research *is to evaluate the neuroprotective effect of citicoline in conditions of acute reversible cerebral ischemia in rats.*

Material and methods. *The study involved 79 male Wistar rats, 65 of which underwent 20-minute subtotal reversible cerebral ischemia by simultaneous bilateral ligation of the internal carotid arteries. The animals were divided into 3 groups: 1st - sham-operated rats; 2nd – rats with ischemia-reperfusion modeling without treatment (control group); 3rd - rats which were administered intravenously once with the drug citicoline immediately after ischemia-reperfusion modeling. The dynamics of lethality and neurological status were assessed. The functional state of the central nervous system was determined using the "open field" test. Changes in biochemical parameters (glucose, lactate, succinate dehydrogenase, malondialdehyde, superoxide dismutase, total NO synthase activity) in the somatosensory cortex were analyzed.*

Results. *In the group 3 mortality was significantly lower than in the control group at the 1th, 12th, and 24th hours of an observation (0%, 24% and 32%). Citicoline significantly reduced neurological deficits in the subacute and recovery periods of the acute ischemic injury (according to the McGraw Stroke-index scale). The citicoline use led to an increase in the duration of the horizontal locomotor activity episodes by an average of 70,0%. Its use also significantly reduced an increase in glucose and lactate levels in the somatosensory cortex on the day 7 by an average of 17,5% and 32,0%, on the day 14 - by 10,1% and 41,1%, when the succinate dehydrogenase level remained 2 times higher, and the malondialdehyde level was lower on average by 55,6% than in rats of the group 2 ($p<0,05$). Also, on the experimental day 7 an increase in the total activity of NO synthase was recorded in the group 3 by an average of 11,1%, and in the group 2 by 82,4%, when on the day 14 – 10,0% and 72.0% ($p<0,05$) respectively.*

Conclusions. *Intravenous administration of citicoline in cerebral ischemia was accompanied by plus dynamics in neurological status, better survival of animals, and normalization of pathobiochemical changes in the somatosensory cortex of a brain in rats, which allows including this agent to secondary neuroprotectors.*

ЗАХИСНИЙ ВПЛИВ ЦИТИКОЛІНУ ПРИ ГОСТРІЙ ОБОРОТНІЙ ЦЕРЕБРАЛЬНІЙ ІШЕМІЇ У ЩУРІВ

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Ключові слова: *ішемія-реперфузія, головний мозок, соматосенсорна кора, нейропротекція, цитопротектори.*

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Резюме. *Реваскуляризаційне втручання при ішемічному інсульті має значні часові та технологічні обмеження. Тому пошук нейропротекторних засобів залишається актуальним.*

Мета роботи *– оцінити нейропротекторний вплив цитиколіну в умовах гострої оборотної ішемії головного мозку в щурів.*

Матеріал і методи. *Для дослідження взяли 79 щурів-самців лінії Вістар, 65 з яких виконали 20-хвилинну субтотальну оборотну ішемію головного мозку шляхом одномоментного двобічного перев'язування внутрішніх сонних артерій. Тварин розподілили на три групи: 1-ша - псевдооперовані щури; 2-га – з ішемією-реперфузією без лікування (контрольна група); 3-тя – котрим одразу після ішемії-реперфузії вводили внутрішньовенно однократно препарат цитиколін. Оцінювали динаміку летальності та*

неврологічного статусу. Визначали функціональний стан центральної нервової системи за тестом «відкрите поле». Аналізували зміни біохімічних показників (глюкоза, лактат, сукцинатдегідрогеназа, малоновий альдегід, супероксиддисмутаза, сумарна активність NO-синтази) у соматосенсорній корі.

Результати. *У групі цитиколіну, порівняно із групою контролю, летальність була вірогідно нижчою в 1-шу, 12-ту та 24-ту год спостереження (0%, 24%, 32%). Цитиколін значно зменшував неврологічний дефіцит у підгострому та відновному періодах гострого ішемічного ураження (за шкалою Stroke-index McGraw). Застосування цитиколіну призвело до збільшення тривалості епізодів горизонтальної локомоторної активності, у середньому, на 70,0 %. Використання цитиколіну достовірно зменшувало зростання рівня глюкози та лактату в ділянці соматосенсорної кори на 7-му добу, у середньому, на 17,5 % та на 32,0 %, на 14-ту - на 10,1 % та 41,1 %, рівень ферменту сукцинатдегідрогенази залишався у 2 рази вищим, а рівень малонового альдегіду був нижчим, у середньому, на 55,6 %, ніж у щурів групи контролю ($p<0,05$). Також на 7-му добу експерименту реєструвалось підвищення сумарної активності NO-синтази у групі цитиколіну, у середньому, на 11,1 %, а в групі контролю - на 82,4 %, на 14-ту - 10,0 % та 72,0 % ($p<0,05$) відповідно.*

Висновки. *Внутрішньовенне введення цитиколіну при ішемії головного мозку супроводжувалось позитивною динамікою змін неврологічного статусу, збільшенням виживаності тварин, нормалізацією патобіохімічних змін у соматосенсорній корі головного мозку в щурів, що дозволяє включити цей засіб до вторинних нейропротекторів.*

Introduction. Ischemic stroke is an episode of acute neurological dysfunction caused by focal cell death in the brain, spinal cord, or retina associated with ischemia (blockage of an artery or vein) [1, https://www.dec.gov.ua/wp-content/uploads/2024/06/2024_-kn-gostryj-ii.pdf]. In Ukraine, since the onset of 2023, acute cerebral stroke has been diagnosed in 87,114 patients, 87,8% of which were ischemic stroke [https://moz.gov.ua/uk/insult-scho-robit-derzhava-dlja-pacientiv]. The world standard in the treatment of ischemic stroke is revascularization intervention after mandatory neuroimaging. This is a high-tech procedure involving a team of highly qualified specialists and a strict time limit (4,5 hours for intravenous thrombolysis and 6 hours for endovascular thrombectomy). Recombinant human tissue-type plasminogen activator (alteplase) or its derivative (tenecteplase) are the only effective agents for pharmacological revascularization in ischemic stroke. However, these drugs have a very short half-life (4-5 min), increase the risk of hemorrhagic transformation, and provoke pro-inflammatory reactions [2]. Therefore, due to the narrow "window of therapeutic opportunity" and numerous contraindications to revascularization, the problem of neuro(cyto)protection remains relevant.

In general, neuroprotection (pharmacological or non-pharmacological) is a means of preventing brain damage in conditions of ischemia [3]. Positive results of combined use of pharmacological neuroprotectors with agents to improve reperfusion have been published [4]. For our own study, among the cytoprotectors available on the Ukrainian pharmaceutical market, we chose citicoline, which has been successfully tested in animal models of ischemia [5, 6, 7], but has shown conflicting results when gone in a clinic [8, 9].

The purpose of the research was to estimate the neuroprotective effect of citicoline in conditions of acute reversible cerebral ischemia-reperfusion in rats.

Material and methods

The investigation is an experimental study made on the basis of the educational-research laboratory for preclinical evaluation of new medications and biologically active compounds "Pharmadar" of Vinnytsia National Pirogov Memorial Medical University (the certificate of technical competence №031/18 until 31.10.2023) in accordance with the State Center of the Ministry of Health recommendations and the bioethics requirements. The research was carried out on 79 sexually mature male Wistar rats bred in the university vivarium at the age of 12-16 weeks, weighing 160-190 g. A 20-minute model of subtotal cerebral ischemia (by simultaneous bilateral ligation of the internal carotid arteries) was selected, because it reproduced acute ischemic type cerebral stroke in the forebrain basin.

The experimental animals were parted into 3 groups. The group 1 included 14 sham-operated rats; the group 2 coupled 40 animals with acute reversible ischemia-reperfusion (IR) without any treatment (control group); the group 3 consisted of 25 rats, which immediately after IR were administered intravenously once with the drug citicoline ("Neuroxon", Arterium Corporation, Ukraine) at a dose of 250 mg/kg.

Alterations in mortality and neurological status were appreciated on the day 7 (correspondes to the subacute ischemic period) and day 14 (correspondes to the recovery period) using the C.P. McGraw stroke-index scale in points

[10]. The functional status of the central nervous system (CNS) was determined using the "open field" test through the behavioral reactions of rats. Changes in biochemical parameters (glucose, lactate, succinate dehydrogenase (SDH), malondialdehyde (MDA), superoxide dismutase (SOD), total NO synthase (NOS) activity) in the somatosensory cortex were assessed.

The Statistica 7.0 program (StatSoft Inc. production, USA) was used to analyze the obtained data. The results were analyzed using nonparametric statistical methods (Mann-Whitney U-test). Differences in mean values of indicators between comparison groups were adviced significant at $p < 0,05$.

Research results and their discussion

Analysis of the mortality in animals that were administered citicoline at a dose 250 mg/kg immediately after acute reversible IR showed significant differences at the 1st, 12th, and 24th observation hours compared to the group 2. Thus, no died animals in the group 3 were during the 1st hour after IR. And in 12 and 24 observation hours after IR, mortality in the group 3 was significantly lower (24% and 32%, respectively), compared to the control group 2 (45% and 65%).

There were no deaths among the sham-operated animals (group 1) (Fig. 1). That is, the collected data indicate the ability of the study medication to increase animal survival.

The study of neurological deficit in sham-operated animals (group 1) showed its absence (fig. 2). In experimental animals of group 2 (control group) acute reversible IR provoked severe neurological changes (paralysis, paresis, ptosis) on the 7th and 14th

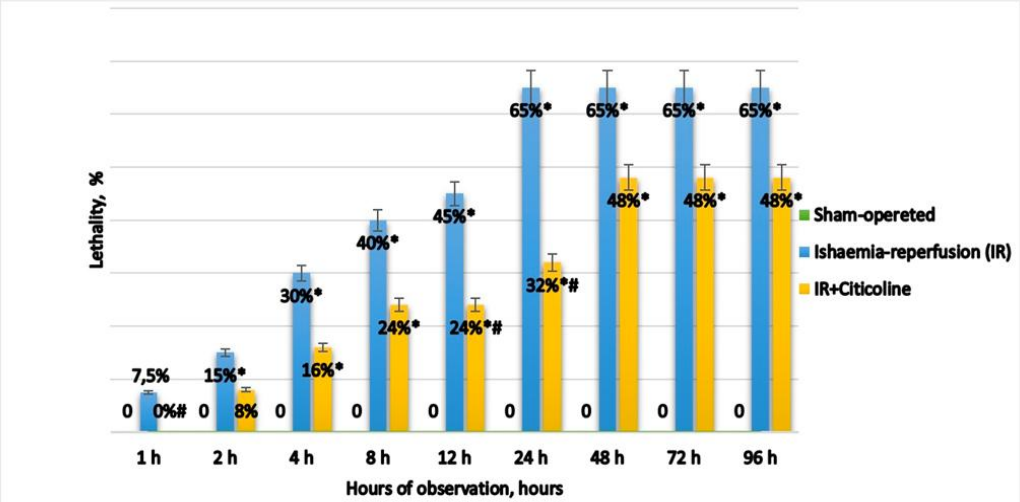

Fig. 1. Effect of intravenous citicoline administration (250 mg/kg) on mortality in rats with acute reversible cerebral ischemia

*Notes: 1. *- p<0,05 relative to sham-operated animals; 2. # - p<0,05 relative to control pathology.*

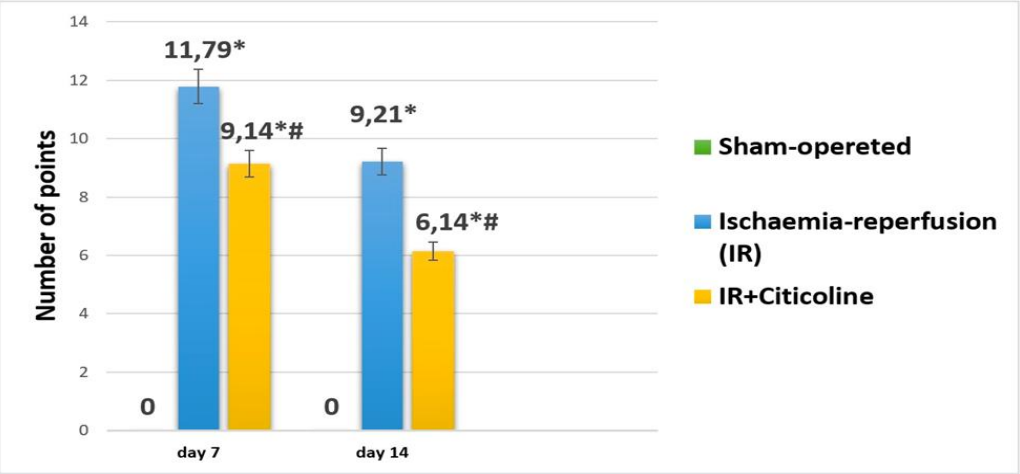

Fig. 2. Effect of intravenous citicoline administration (250 mg/kg) on the neurological deficit dynamics in obedience to the McGraw Stroke-index scale

*Notes: 1. * - p<0,05 in relation to the indicator of sham-operated rats; 2. # - p<0,05 in relation to the indicator of control pathology.*

experimental days. After a single intravenous citicoline administration, a significant regression of neurological deficit was observed. Moreover, the most significant differences were determined in the recovery period (on the 14th day after IR) and amounted to 6,14±0,15 points versus 9,14±0,15 points in the group 2 (p<0,05). Thereof, the data obtained point out citicoline significantly reduces neurological deficits in the subacute and recovery periods of acute ischemic injury.

The citicoline influence on behavioral responses in rats after acute cerebral IR was assessed by the "open field" test (3 min. observation). This method allows analyzing animal behavior by assessing emotional-behavioral reactivity, dynamics of individual behavioral elements, and locomotor stereotypy.

The use of citicoline led to an increase in the duration of horizontal locomotor activity episodes by an average of 70,0% (p<0,05), compared with the group having control pathology. On the day 14 there was detected a tendency to diminish the indicators of horizontal motor activity in the group 2 (rats with control pathology) after their testing in the "open field" in comparison with the results gotten on the day 7. If using citicoline, an increase in the duration of spontaneous horizontal motor activity episodes was observed, that confirms the neuroprotective effect of the drug on the affected IR areas of a brain.

The neuroprotective effect of the studied agent is closely related to its effect on brain metabolism [11-13]. Analysis of some biochemical parameters gave us the possibility to assess citicoline influence on glucose metabolism, oxidative and nitrosative stress indicators (Fig. 3).

On the day 7 a rise in glucose content was noted in the 2nd and 3rd groups of rats by 51.2% and 17.5% (p<0,05) relative to sham-operated animals (Fig. 3). However, in the rats treated with citicoline the elevation of the indicator was significantly lower (17,5%, p<0,05). In the recovery period (day 14) the changes were similar, but less pronounced (Fig. 4). The glucose content in the group 2 and group 3 increased on average by 33,9% and 10,1%, respectively. It is known that systemic hyperglycemia in cerebral infarction promotes glucose uptake into ischemic tissue. On the other hand, there is compensatory activation of the anaerobic glucose metabolism pathway and an elevation in lactate and hydrogen ions which causes the appearance of metabolic acidosis [14].

With regard to the lactate content, on the day 7 after IR its level increased on average by 305,6% in the group 2 and by 175,8% in the group administered with citicoline (fig. 3). It should be noticed the lactate content in the group 3 was lower significantly by 32,0%. On the day 14 the high content of the indicator remained in both the groups (fig. 4). However, citicoline therapy demonstrated a positive effect on metabolic imbalance: lactate content in the group 3 was 41,1% lower in comparison to the group 2. Whereas lactate is an extra energy substrate for nervous cells, the results obtained indicate a normalizing effect of citicoline on the appearance of decompensated lactic acidosis in cerebral tissues.

Succinate dehydrogenase (SDH) is a key enzyme in the Krebs cycle. In rats of the group 3 on the 7th day its content amounted 6,41±0,38 μmol/min.mg protein in the brain somatosensory cortex, which was 21,4% less than in sham-operated animals (p<0,05), but 2 times higher than in rats of the group 2 (p<0,05) (fig. 3). On day 14 its content in the group 3 decreased by only 5,6% compared to rats of the group 1 and remained significantly higher (by 97,5%) compared to the control (Fig. 4).

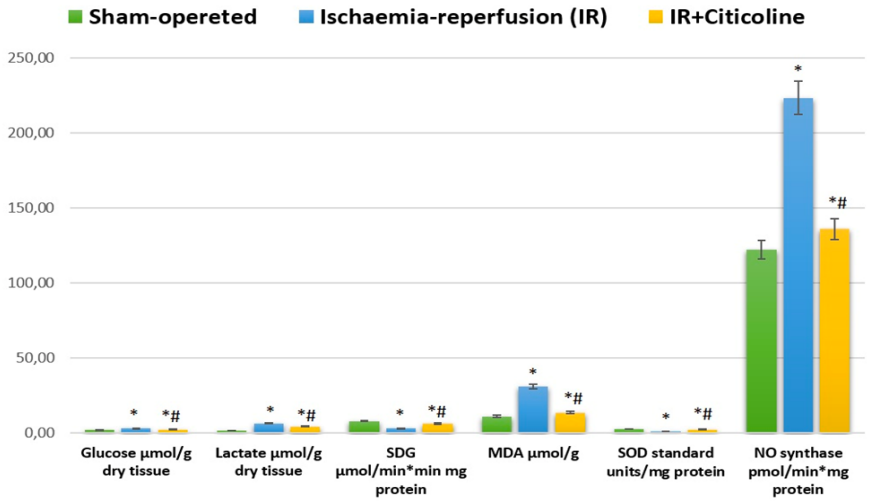

Fig. 3. Effect of intravenous citicoline administration (250 mg/kg) on biochemical indicators in the rat somatosensory cortex on the day 7 after cerebral ischemia-reperfusion

*Notes: **

*1. * - p < 0,05 relative to the corresponding group of sham-operated animals;*

2. # - p < 0,05 relative to the corresponding group of animals with control pathology

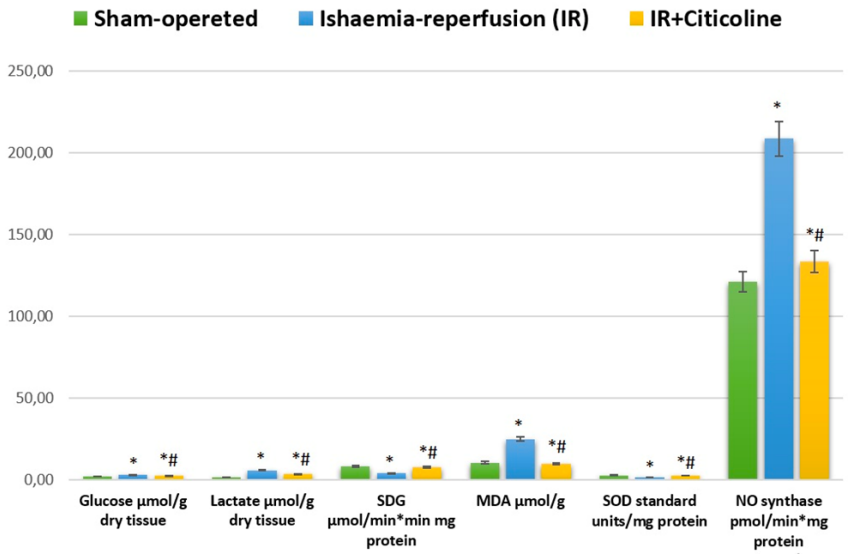

Fig. 4. Effect of intravenous citicoline administration (250 mg/kg) on biochemical parameters in the somatosensory cortex of rats in conditions of cerebral ischemia-reperfusion on the 14th study day

Notes:

*1. * - p < 0,05 relative to the corresponding group of sham-operated animals;*

2. # - p < 0,05 relative to the corresponding group of animals having control pathology.

Clear differences were recorded in MDA content analysing on days 7 and 14. On day 7 in the rat somatosensory cortex of both the control and citicoline groups the MDA content rise was recorded, which shown oxidative stress (fig. 3). However, when comparing the group 3 with the group 2 the MDA level was significantly lower on average by 55,6% ($p<0,05$). On day 14 an elevation of this indicator was observed only in the control rats (fig. 4).

Recent publications prove an important role of active nitrogen forms in the pathological changes' development in cerebral IR [15, 16]. One of the remarkable mechanisms of the protective action of modern neuroprotectors is their corrective effect on NO metabolism, in particular on the nitrosative stress development in brain tissues [17].

The study found that brain IR in rats leads to the total NOS activity increase in in the somatosensory cortex on the day 7 both in the group 2 and in the group 3 by an average of 82,4% and 11,1% respectively ($p<0,05$). It should be emphasized that these changes were minimal in the rats administered with citicoline (Fig. 3). As of day 14, the NOS elevation was 72,0% and 10,0% ($p<0,05$) compared with sham-operated rats (Fig. 4). That is, the depressing effect on the total NOS activity occurred when using citicoline. Therefore, citicoline contributed to the restoration of normal functioning of the NO cycle in the ischemic rat brain both in the subacute and recovery periods of stroke.

Conclusions

1. In a model of 20-minute reversible bilateral internal carotid arteries occlusion in rats it was detected ischemic-reperfusion injury of the brain is accompanied by a significant shift in biochemical parameters with the progression of energy deficiency, oxidative and nitrosative stress, and acidosis, which led to an increment in neurological deficit and mortality of experimental animals.

2. Intravenous citicoline administration at a dose of 250 mg/kg in simulated brain ischemia-reperfusion was accompanied by plus dynamics of changes in neurological status and increased survival of animals, as well as normalization of pathobiochemical alterations in the somatosensory cortex in the rat brain.

3. Considering the identified mechanisms of protective action of citicoline on ischemic neurons of a brain, this medication can be classified as a secondary neuroprotector.

Prospects for further research

A further research is aimed at studying the cerebroprotective influencess of mesenchymal stromal cells having different origin to compare its action with citicoline's in acute IR brain injury.

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