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Ischemic morphological damage improvement by citicoline in rat brain

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In spite of wide range of pharmacological agents investigated for protection of neuronal cells from ischemic damage, only limited structures have revealed effectiveness for stroke treatment [10, 18]. In view of this, the investigation and development of neuroprotective drugs as defensive compounds for nervous tissue remain as one of the important tasks of neurology [4].

The recent investigations have given evidence that one of the most important sources for pharmacological improvement of ischemic damage are endogenous neurochemical components of brain, including neuropeptides [7], neuroactive amino acids [12, 14], neurohormones [15], phospholipids [13, 19] etc. Citicoline, the endogeneous ether of cytidine and choline, has been among attractive investigated substances in the last few decades. It is known that citicoline is a safe drug (LD50 value for rats is 4,150 mg/kg) [9] approved for the treatment of acute stroke due to its ability to enhance neurological and functional recovery after ischemic brain damage [1- 3]. The possible mechanisms of its neuroprotective action were postulated to be: (a) repair of the neuronal membrane via increased synthesis of phosphatidylcholine; (b) repair of damaged cholinergic neurons via potentiation of acetylcholine production; and (c) reduction of free fatty acid buildup at the site of stroke-induced nerve damage [8, 16]. That is why neuroprotective effects of citicoline have remained the object of remarkable interest for scientific investigation as the exact mechanism of the mentioned activity remains still unknown [6, 11].

Thus, the aim of the presented study was investigation of citicoline ability to prevent the morphological changes typical of ischemic disturbances.

Materials and Methods

White inbred male rats, weighing 180 to 200 g, were used in the

experiments. All rats were kept under controlled environmental conditions (ambient temperature 22°C, 12/12-hour light/dark cycle, lights on at 7:00 AM). Standard laboratory chow and tap water were given ad libitum. The study was conducted according to the principles of the Guide to the Care and Use of Laboratory Animals [21].

Local ischemia was obtained by the middle cerebral artery occlusion (MCAO) by the method of Tamura et al. [20], modified by Topchian et al. [22] with use of chloral hydrate anesthesia (400 mg/kg, i/p).

Animals were divided into three groups. In the first and second experimental groups rats with MCAO received citicoline (*Somazina, Ferrer International*) immediately after occlusion in a dose of 12.5 mg/kg twice per day during 6- and 12 days, respectively. The third control group of animals received normal saline under the same conditions. Dose of citicoline was chosen based on clinical trials [5].

For histological analysis, sections of the brain tissue were taken 6 and 12 days after MCAO [17]. The preparations were stained with hematoxylin-eosin according to the standard operating protocol. After decapitation, the rat brain was extracted under cold conditions (on ice) and immediately fixed in a 10% buffered formalin neutral solution. 24 hours after fixation, a frontal dissection of the brain tissue was carried out in the projection of the pool of the middle cerebral artery. Sliced pieces were embedded to paraffin to form paraffin blocks, from which serial sections with a thickness of 4 µm were obtained.

Results and Discussion

The investigation has shown that daily intraperitoneal citicoline injection in the dose of 12,5 mg/kg causes decreasing of morphological changes after MCAO in rat brain. The study of structural changes by staining of the frontal sections of the brain made it possible to detect tissue changes in the regions of both left and right middle cerebral arteries and to characterize the comparative morphological changes of occluded (left) and not occluded (right) hemispheres, as well as the degree of its depending on the duration of the MCAO – induced ischemia.

The time intervals for the rat brain histopathological analysis were selected based on the evaluation of the affected area in the chosen model, performed by Topchian AV et al. [22] according to which the formation of ischemic focus is observed on the 6th day after MCAO. To characterize the long-term results, sections of the brain tissue were also analyzed on the 12th day after occlusion.

The morphological examination of the sections of brain tissue isolated on the 6th day after MCAO showed that in control group of animals local ischemia was accompanied by atrophic changes in the ipsilateral hemisphere Caudate-putamen complex (CPu), Globus pallidus (GP) zone of cortex (Fig. 1) with

signs of pyknosis of neurons in the same zone. In the contralateral hemisphere with a preserved structure, hyperemia of the vessels of the microcirculatory bed was observed.

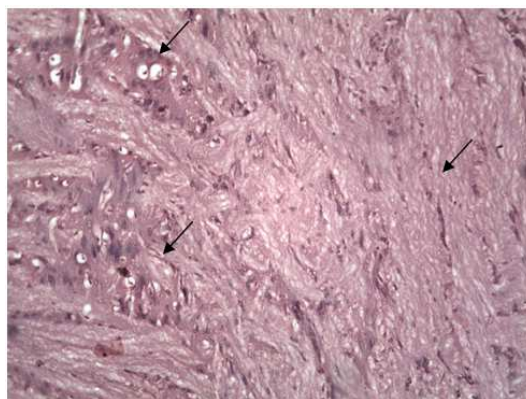


Fig. 1. Pyknosis of neurons of ipsilateral CPu and GP zone of cortex on the 6th day after MCAO (hematoxylin-eosin $\times 400$)

The study results have shown that in case of increasing the occlusion duration up to 12 days similar morphological changes were observed. In response to ischemia a necrosis of brain cortex in Par1 and Cg1 fields have been noticed. Local ischemia in the ipsilateral hemisphere of rats after 12 days MCAO was accompanied also by focus of hemorrhage with hemosiderosis in the area of frontal amygdaloidal field with the glial rough scars formation in the area of the ipsilateral CPu and GP, outer capsule. A marked venous vascular dystonia was also noted (Fig. 2). Cortical arteries were "empty", convolutive. Hyperchromatosis with focal pyknosis of neurons in the cortical fields – Par1 and Cg1 of the ipsilateral hemisphere was mentioned. The structure of right hemisphere was retained.

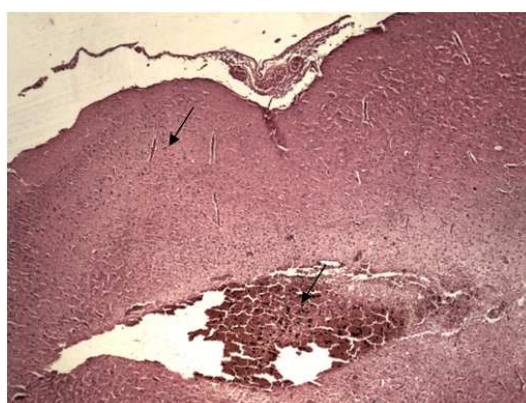


Fig 2. Venous vascular dystonia and haemorrhage, hyperchromatosis with focal pyknosis of neurons in the cortical fields – Par1 and Cg1 of the ipsilateral hemisphere on the 12th day after MCAO (hematoxylin-eosin, $\times 200$)

In comparison with the control group in the experimental group of animals receiving citicoline after 6-days of MCAO we have noticed comparable recovery of histostructure of rat brain tissue. Proliferation of glial cells in subcortical layer was observed. Though, a symmetric hyperaemia of brain capillaries, pyknosis of ipsilateral cortex were seen (Fig. 3).

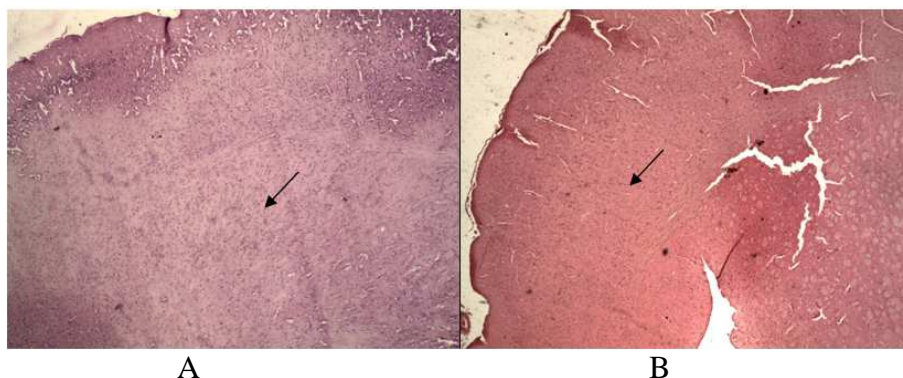


Fig. 3. Proliferation of glial cells of subcortical layer and pyknosis of ipsilateral cortical fields—Par1 and Cg1 on the 6th day after MCAO with intraperitoneal citicoline injection (hematoxylin-eosin, $\times 200$ (A) and $\times 400$ (B))

More significant recovery changes have been observed in the experimental group of rats after 12-days MCAO compared with 6-days ischemia. Evident gliosis in the areas of CPu, GP of ipsilateral hemisphere with proliferation of vessels without rough scarring was stated. There were not any signs of vascular disorders in the ipsilateral and contralateral areas. There was also noted a prevention of neuronal damage in the Par1 and Cg1 fields of ipsilateral hemisphere. The cortical layer was preserved. The ventricles of hemisphere were symmetric (Fig. 4).

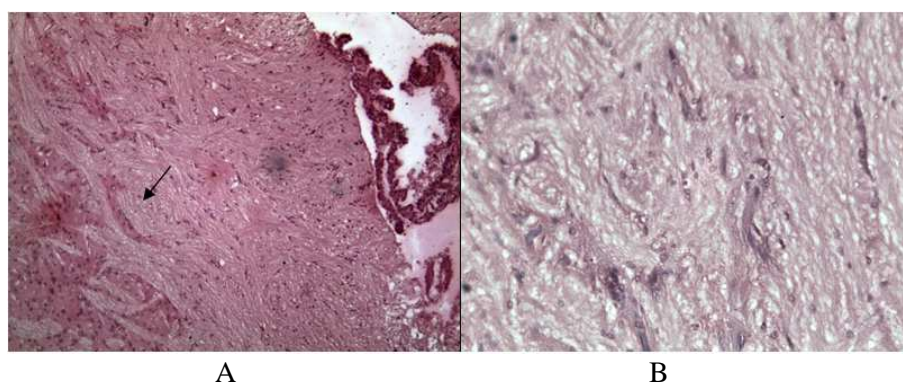


Fig. 4. Gliosis without rough scarring in the areas of CPu, GP of ipsilateral hemisphere with proliferation of vessels on the 12th day after MCAO with intraperitoneal citicoline injection (hematoxylin-eosin, $\times 200$ (A) and $\times 400$ (B))

Thus, the morphological investigation gives evidence that citicoline has ability to protect brain tissue from ischemic damage, as morphological changes caused by MCAO were prevented both after 6th and especially 12th days of intraperitoneal injection of the mentioned compound. The obtained results could serve as evidence-based data for the neuroprotective efficacy of citicoline.

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Իշեմիկ մորֆոլոգիական ախտահարումների բարելավումը առնետների մոտ ցիտիկոլինի ազդեցությամբ

Տ.Ս. Գրիգորյան, Ա.Վ. Պապյան, Մ.Գ. Բալասանյան

Ստացված արդյունքները վկայում են, որ ցիտիկոլինը կանխարգելում է լոկալ իշեմիայի արդյունքում առաջացած մորֆոլոգիական փոփոխությունները, հարուցված միջին ուղեղային զարկերակի կապումով (ՄՈԻԶԿ): Ցիտիկոլինի 6 և հատկապես 12 օր տևողությամբ ներորովայնային ներարկումը ուղեկցվում է ՄՈԻԶԿ-ով հարուցված հյուսվածքաախտաբանական փոփոխությունների կանխարգելմամբ:

Այսպիսով, իրականացված հետազոտության արդյունքները ցույց տվեցին, որ ցիտիկոլինը պաշտպանում է ուղեղային հյուսվածքը իշեմիայից: Հետազոտության արդյունքները կարող են ծառայել որպես ցիտիկոլինի նոր նյարդապաշտպան ազդեցության արդյունավետության ապացույց:

Улучшение ишемических морфологических повреждений под влиянием цитиколина у крыс

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Полученные данные свидетельствуют о способности цитиколина устранять морфологические изменения локальной ишемии, вызванные окклюзией средней мозговой артерии у крыс (ОСМА). Внутривентрикулярная инъекция цитиколина в течение 6 и, особенно, 12 суток сопровождается предотвращением морфологических изменений, вызванных ОСМА.

Таким образом, морфологические исследования показали, что цитиколлин защищает головной мозг от ишемии. Результаты исследования могут служить доказательством новой нейропротективной эффективности цитиколина.

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