Cerebroprotective Effects of Diquertin and Ascorbic Acid

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Electron microscopy and electrophysiological studies revealed pronounced structural and functional changes in the brain cortex in rats with experimental cerebral ischemia. Repeated administration of diquertin and ascorbic acid significantly attenuates ischemic damage induced by circulatory disturbances.

Key words: *diquertin; ascorbic acid; brain ischemia; cortical neurons; electroencephalogram*

Disturbances of cerebral hemodynamics are a severe and prevalent disorder associated with frequent disabling and high mortality and therefore presents an important medical and social problem [6]. The efficacy of specific drug therapy decreasing mortality and promoting recovery is not high [11,12]. Therefore, the search of new cerebroprotective compounds for pathogenetic therapy of brain circulation disturbances is an important trend of morden pharmacology. Since lipid peroxidation (LPO) plays a key role in brain cell damage [1], antioxidant compounds are of special interest. Diquertin possesses pronounced antioxidant properties [10], which are potentiated by ascorbic acid (AA) [3]. The aim of the present study was to examine cerebroprotective effects of a diquertin-AA mixture on the model of rat cerebral ischemia (CI).

MATERIALS AND METHODS

Morphological studies were carried out on 28 adult Wistar rats of both genders weighing 250-300 g kept on standard vivarium nutrition. Intact rats (n=10) presented group 1, in groups 2 (n=10) and 3 (n=8) rats CI was modeled by ligation of the left carotid artery and 50% restriction of the blood flow via the right carotid artery [8]. Group 3 rats daily received diquertin-AA mixture (20 and 50 mg/kg, respectively) in 1% starch mucus through a gastric tube, for 5 days starting from day 1 after CI. Group 2 rats (control) received the same volume of 1% starch mucus. The animals were decapitated under ether anesthesia on day 5 after CI. The right and left brain hemispheres were fixed in Carnoy fluid and embedded in paraffin. Frontal deparaffinated sections on the level of the anterior parietal area (PA_5) of the brain cortex were stained with hematoxylin and eosin and cresyl violet according to Nissl. Morphologically altered neurons per 500 neuronal cell in layers III, IV and V were counted. The damage and reaction of cortical neurons to ischemia and diquertin-AA treatment were estimated by standard neuromorphological criteria: focal chromatolysis and hyperchromia without shrinkage were regarded as reversible changes, while total chromatolysis associated with the formation of ghost cells, hyperchromia and shrinkage were considered as irreversible neuronal changes [2,5].

For electron microscopy, the samples of brain cortex from the anterior parietal area were fixed in 2.5% glutaraldehyde cacodylate buffer (pH 7.4), postfixed in 1% OsO_4, dehydrated in alcohol and embedded in araldite. Semithin sections were stained with toluidine blue, ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM-7A electron microscope. The results were statistically processed using Mann—Whitney nonparametric test.

The effects of test drugs on brain activity were estimated by compressed spectral array of EEG. EEG was recorded via intracortically implanted and com-

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pletely insulated (except butt-ends) Ni-Cr electrodes connected to an Era-9 electroencephalograph and magnitograph 1296 (OTE-Biomedica); Fourier analysis was performed on a spectroanalyzer of the same company. The epoch of the analysis was 248 sec. According to recommendations of International Federation of the Electroencephalography and Clinical Neurophysiology Societies, the EEG spectrum was divided into 5 initial bands for quantitative analysis.

RESULTS

On day 5 after CI modeling we observed pronounced edema of astroglia along partly constricted or completely closed venules and arterioles (Fig. 1, *a*). Focal chromatolysis in neuronal perikaryon was manifested as peripheral or segmentary dissociation of chromatin, which was regarded as the result of long-term functional strain of a neuron and presented a standard reaction to oxygen deficiency [2,5]. The number of neurons with focal chromatolysis increased significantly compared to intact animals especially in the left hemisphere (Table 1). Similar dynamics was observed for the number of hyperchromic nonshrinked neurons morphologically identified by increased basophilia of the perikaryon and enlarged chromatin conglomerates. This was functionally interpreted as reactive inhibition of a neuron [7]. The number of ghost cells resulting from irreversible chromatolysis associated with complete chromatin disappearance in the perikaryon and dendrites and sharp reduction or disappearance of the nucleoli increased in the right and, especially, in the left hemispheres. The content of irreversibly changed shrunken neurons also increased, while the number of unchanged normochromic cells significantly decreased (Table 1). These morphological changes indicate pronounced oxygen deficiency [2,5].

On day 5 of ischemia, EEG showed signs of pronounced cerebral hypoxia [4]. Total EEG power in the parietal cortex decreased significantly both in the left and right hemispheres (by 37 and 27%, respectively). Normally dominant θ-rhythm was inhibited by 70 and 44% in the left and right hemispheres, respectively; the asymmetry coefficient for θ-rhythm was 0.51. δ-Rhythm decreased by 49 and 40% in the left and right hemispheres, respectively (Fig. 2).

Repeated administration of diquertin and AA decreased ischemia-induced damage to cortical cells, this effect depended on the degree of cell damage and CI severity and showed interhemispheric differences (Table 1). In particular, in the right hemisphere affected by moderate ischemia the number of cells with focal chromatolysis decreased approaching the level of intact animals. Therapy significantly decreased the number of irreversibly changed neurons (ghost cells and pyknomorphic neurons). In the left hemisphere with more pronounced ischemic damage, the number of reversibly changed neurons decreased. The content of irreversibly altered cells remained unchanged, however, the number of normochromic neurons increased compared to the control. The dynamics of the content of morphologically changed neurons in layers III and IV during ischemia and against the background of diquertin and AA was similar to that in layer V (data not shown).

Ultrastructural CI-induced changes were manifested as chromatolysis, decreased content of granular endoplasmic reticulum and free ribosomes in the perikaryon, vacuolation and decreased electron density of the cytoplasm (Fig. 1, *b*). In group II rats specific volume of granular endoplasmic reticulum in the right and left hemispheres decreased by 46 and 69%, respectively, while in animals treated with diquertin and AA this parameter decreased by 16 and 27%, respectively. In animals with CI specific volume of lysosomes and lipofuscin increased by 10.7±1.9 (compared to 2.1±0.4% in the control) and 14.9±2.7% (compared to 1.8±0.5% in the control; $p<0.05$) in the right and

TABLE 1. Effect of Course (5 Days) Administration of Diquertin and AA on the Neurons of Layer V of Rat Cortex after CI (% Cells, *M±m*)

Neurons	Intact		Ischemia			
			control		experiment	
	LH	RH	LH	RH	LH	RH
Normochromic	87.56±1.36	83.75±2.43	34.38±3.19*	59.69±1.95*	53.68±3.21*+	71.79±1.79*+
With focal chromatolysis	4.80±0.26	5.00±0.43	10.51±0.60*	7.03±0.30*	7.40±0.61*+	4.86±0.32+
Hyperchromic without shrinkage	3.50±0.32	4.66±1.08	25.50±0.92*	15.70±1.04*	13.88±1.04*+	9.76±0.87*+
Total chromatolysis (ghost cells)	1.67±0.24	3.41±0.32	13.70±1.63*	8.41±0.25*	9.94±0.65*	6.31±0.25*+
Hyperchromic shrunken	2.47±0.53	3.18±0.58	15.86±0.91*	9.17±0.37*	15.1±0.9*	7.28±0.38*+

Note. $p<0.05$ compared to intact (*) and control (+) animals. LH: left hemisphere, RH: right hemisphere.

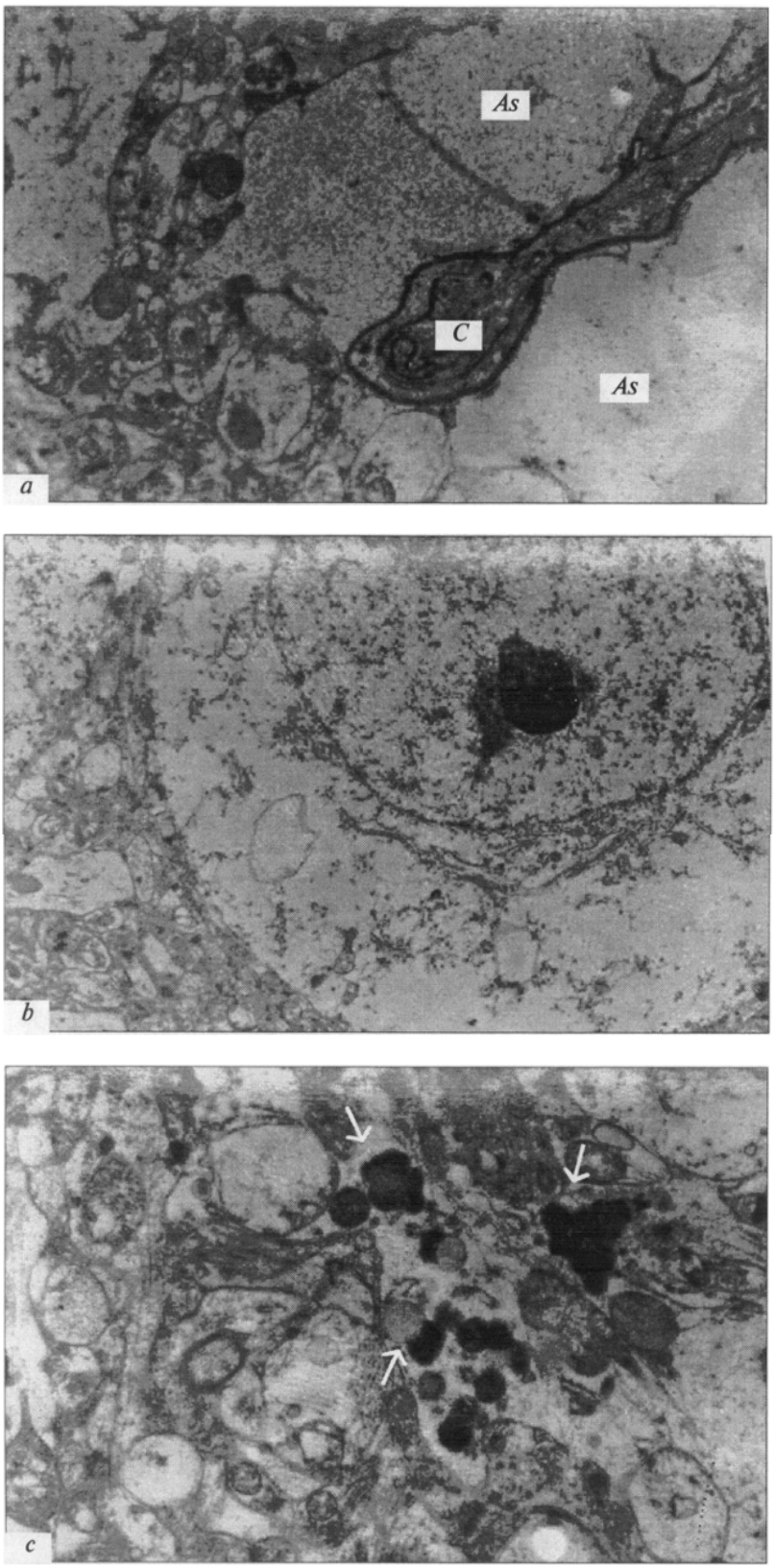

Fig. 1. Ischemia-induced ultrastructural changes in the anterior parietal cortex of rat brain, ×8000. *a*) pronounced edema of vascular astrocyte processes (*As*) compressing the capillary (*C*); *b*) pronounced chromatolysis, vacuolation of neuronal perikarion; *c*) accumulation of lipofuscin and lipids (arrows) in neuronal processes.

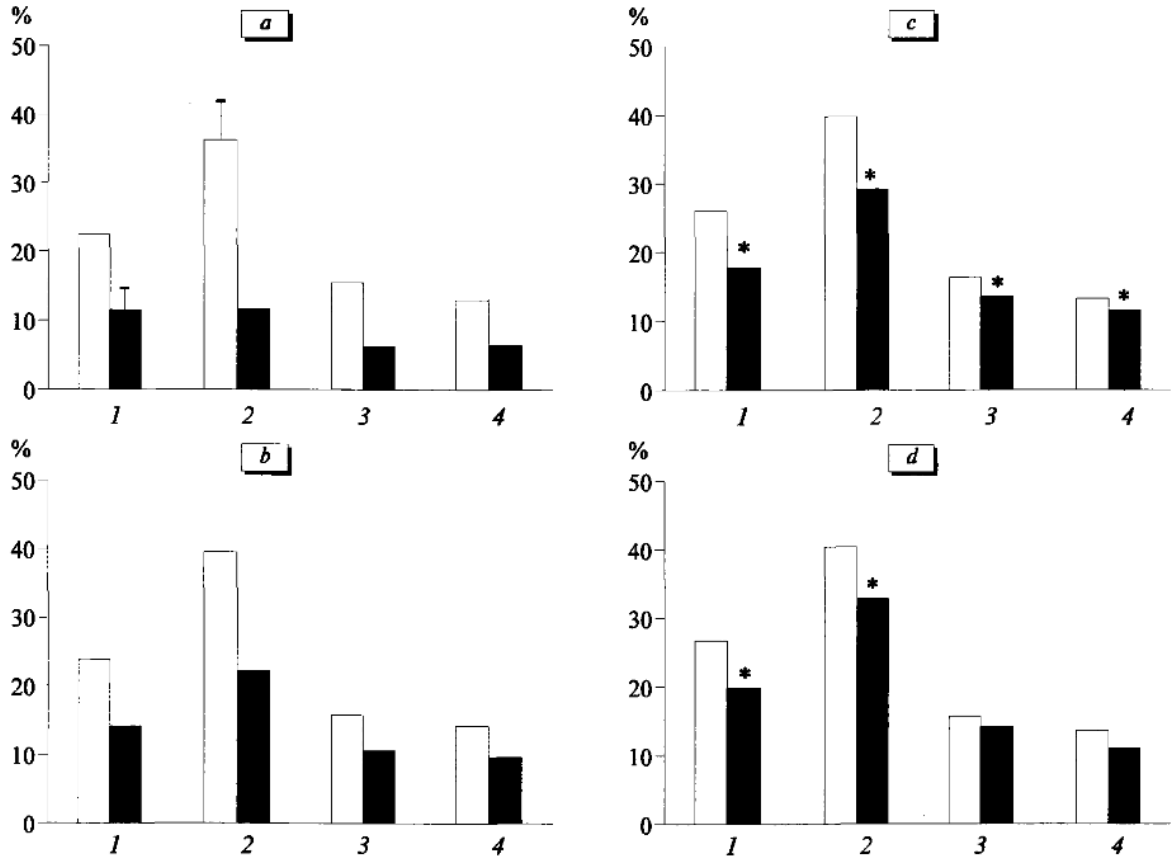

Fig. 2. Power of δ- (*1*), θ- (*2*), α- (*3*), and β- (*4*) rhythms of EEG spectrum in CI (*a*, *b*) and against the background of repeated administration of diquertin and ascorbic acid (*c*, *d*). *a*, *c*) left, *b*, *d*) right hemisphere. Open columns — initial level, dark columns — day 5 after ischemia. *$p<0.05$ compared to the control.

left hemispheres, respectively, while in animals treated with diquertin and AA these parameters increased less markedly (9.4±0.9 and 9.5±2.1%, respectively).

Diquertin and AA therapy significantly improved functional activity of the brain cortex: the power of δ-, θ-, and β-rhythms in the left hemisphere increased by 54, 54, and 78%, respectively, compared to the control. In the right hemisphere the power of δ- and θ-rhythms was close to that of intact animals. Interhemispheric EEG asymmetry decreased (Fig. 2).

Thus, the presented functional and morphological data confirm potent cerebroprotective activity of diquertin and AA during CI.

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