Cerebroprotective Effect of a New Taurine Derivative during Cerebral Ischemia

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A new taurine derivative chlorohydrate-N-isopropylamide-2-(1-phenylethyl)aminoethanesulfonic acid normalized energy metabolism, inhibited lipid peroxidation, and reactivated antioxidant enzymes in the brain of rats exposed to ischemia. This taurine derivative decreased the mortality rate of animals with ischemic changes in cerebral circulation. The test compound was more potent than piracetam in producing the cerebroprotective effect.

Key Words: *cerebral ischemia; antihypoxants; antioxidants; chlorohydrate-N-isopropylamide-2-(1-phenylethyl)aminoethanesulfonic acid; piracetam*

Neuroprotective drugs providing metabolic protection of the brain play an important role in the therapy for acute cerebral circulation disturbances of the ischemic type. These drugs include antihypoxants and antioxidants that correct changes in energy production and lipid peroxidation (LPO) [8].

Original compounds of endogenous taurine metabolite 2-aminoethanesulfonic acid were synthesized at the Department of Neuropharmacology (Institute of Experimental Medicine, Russian Academy of Medical Sciences). These substances are presented by N-phenylalkyl derivatives of taurine with normal- or branched-chain. Screening revealed chlorohydrate-N-isopropylamide-2-(1-phenylethyl)aminoethanesulfonic acid (TAU-15) exhibiting antihypoxic activity under conditions of normobaric hypoxia accompanied by hypercapnia and hemic hypoxia. This drug produces an antioxidant effect during toxic liver injury and myocardial ischemia [11,12].

Here we studied the effects of TAU-15 on energy metabolism, LPO, and enzyme activity of the antioxidant system (AOS) during cerebral ischemia. We estimated the mortality rate of animals with ischemic disturbances of cerebral circulation. Piracetam served as the reference drug.

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MATERIALS AND METHODS

Experiments were performed on 210 male outbred albino rats weighing 180-200 g. Cerebral ischemia was modeled by occlusion of the common carotid arteries for 1.5, 24, and 72 h. The animals were divided into 4 groups: sham-operation, ischemia+physiological saline (control), ligation of carotid artery+25 mg/kg TAU-15, ligation of carotid artery+100 mg/kg piracetam. The test drugs and placebo were injected intraperitoneally. The first injection was performed 30 min after occlusion. Further injections were performed daily until the end of study. The concentrations of lactate and pyruvate [15], lipid hydroperoxides [9], and malonic dialdehyde (MDA) [10] and activities of superoxide dismutase [6] and catalase [13] were measured in homogenates of cerebral hemispheres.

A special series was conducted to estimate the 14-day survival rate and median survival time (time-to-survival of 50% animals). The relative effectiveness of the test drug was calculated as follows [7]:

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$$Z=(D-C)/(1-C),$$

where Z is the relative effectiveness; D is the survival rate of drug-receiving animals; and C is the survival rate of control animals.

The results were analyzed routinely by Student's *t* test (Statgraphics software).

RESULTS

Common carotid artery occlusion for 1.5 h increased brain lactate concentration by 160% and decreased pyruvate concentration by 42% compared to sham-operated animals (Table 1). The lactate/pyruvate ratio serves as a criterion for aerobic or glycolytic conversion of carbohydrates. The increase in this ratio from 9.8 to 44.3 (by 4.5 times) reflected inhibition of aerobic pathways and activation of "emergency" glycolytic pathways for energy production. The intensity of glycolysis decreased with increasing the duration of occlusion to 24 to 72 h. These changes manifested in a decrease in the lactate/pyruvate ratio to 22.2 and 13.2, respectively.

Impairment of tissue respiration during hypoxia leads to activation of free radical processes [5]. The concentrations of lipid hydroperoxides and MDA in the brain increased by 37 and 35%, respectively, after 1.5-h cerebral ischemia. The observed changes were accompanied by a decrease in SOD and catalase activities (by 26 and 22%, respectively). These enzymes play a key regulatory role at the initial stages of LPO. These interrelated enzyme systems suppress generation of hydroxyl radicals interacting with cell membrane lipids [8].

The intensity of LPO increased, while activity of endogenous AOS decreased after 24-h ischemia. The concentrations of lipid hydroperoxides and MDA increased by 42 and 89%, respectively. Activities of SOD and catalase decreased by 35 and 31%, respectively. Production of oxygen radicals decreased, while antioxidant enzyme activity increased after 72-h occlusion (compared to 24-h ischemia). However, the test parameters did not reach the baseline level.

TAU-15 improved energy metabolism in the ischemic brain. The test drug activated aerobic oxidation of carbohydrates 1.5 h after the start of carotid artery occlusion. This substance was more potent than piracetam in producing the positive effect on energy production. TAU-15 and piracetam decreased the lactate/pyruvate ratio from 44.3 to 13.0 and 17.3, respectively.

The concentrations of lactate and pyruvate in animals receiving the test drugs and exposed to

TABLE 1. Effect of Drugs on Metabolism in the Brain of Rats with Ischemia ($M\pm m$, n=8-10)

Parameter	Sham-operated	Ischemia								
		1.5-h			24-h			72-h		
		placebo	TAU-15	piracetam	placebo	TAU-15	piracetam	placebo	TAU-15	piracetam
LA, μmol/g	2.55±0.04	6.65±0.16*	2.73±0.08+	3.47±0.07+	3.99±0.08*	2.34±0.05+	2.41±0.04+	2.24±0.05*	2.27±0.06	2.32±0.11
PA, μmol/g	0.26±0.02	0.15±0.01*	0.21±0.02+	0.20±0.02+	0.18±0.01*	0.25±0.02+	0.25±0.03+	0.17±0.01*	0.25±0.03+	0.25±0.03+
LA/PA	9.8	44.3	13.0	17.3	22.2	9.4	9.6	13.2	9.1	9.3
LHP, OD_{480}	0.097±0.004	0.133±0.002*	0.102±0.006+	0.112±0.003+	0.138±0.007*	0.085±0.003+	0.098±0.005+	0.121±0.004*	0.094±0.001+	0.098±0.004+
MDA, nmol/g	7.39±0.34	9.98±0.19*	7.48±0.45+	8.18±0.25+	13.97±0.36*	5.03±0.40+	7.26±0.67+	10.35±0.43*	7.11±0.26+	7.38±0.47+
SOD, A/mg protein	3.48±0.12	2.57±0.21*	3.57±0.28+	3.37±0.18+	2.26±0.05*	3.19±0.13+	3.12±0.10+	2.89±0.12*	3.58±0.09+	3.32±0.09+
Catalase, μmol H_2O_2/mg protein/min	7.35±0.31	5.73±0.27*	7.28±0.18+	6.93±0.26+	5.07±0.21*	7.00±0.26+	6.84±0.24+	5.88±0.21*	7.45±0.21+	6.88±0.25+

Note. p<0.05: *compared to sham-operated rats; +compared to placebo.

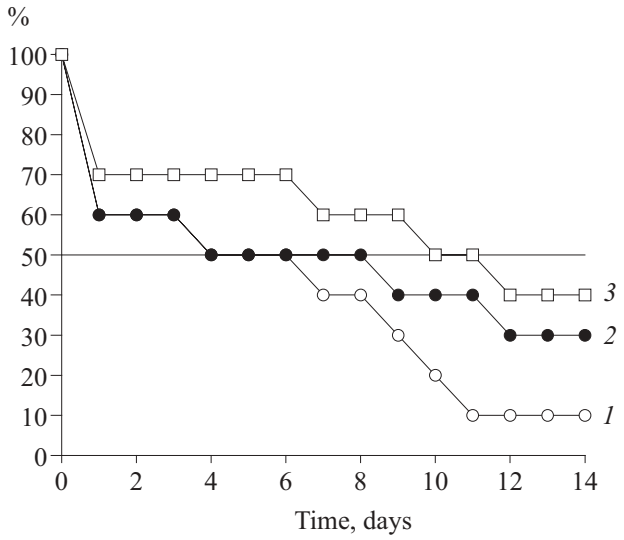

Fig. 1. Survival rate of rats with cerebral ischemia: control (*n*=30, *1*); piracetam (*n*=30, *2*); TAU-15 (*n*=30, *3*). Solid horizontal line, median survival time.

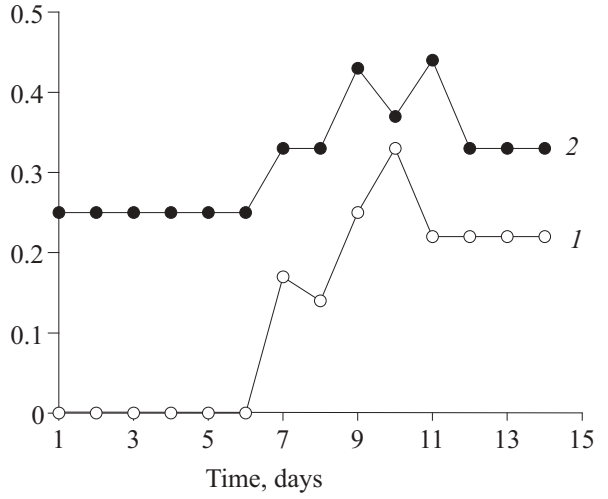

Fig. 2. Relative effectiveness of drugs: piracetam (*1*) and TAU-15 (*2*).

ischemia for 24 and 72 h did not differ from the baseline level. These results attest to normalization of impaired energy metabolism.

TAU-15 prevented hyperactivation of LPO under conditions of ischemia for 1.5-72 h. The positive effect of TAU-15 was most significant during 24-h ischemia. The concentrations of lipid hydroperoxides and MDA in rats with 24-h ischemia were much lower than in sham-operated animals.

TAU-15 restored enzyme activity of AOS at various stages of ischemia. The test parameters in treated rats did not differ from those in sham-operated animals. These data indicate that the test drug is involved in the regulation of hydroxyl radical generation during the initial stages of LPO. Our previous studies showed that TAU-15 activates the antioxidant glutathione system degrading organic peroxides [11].

Piracetam had a positive effect on LPO and improved the state of exhausted AOS in various stages of cerebral ischemia. However, piracetam was less potent than TAU-15 in preventing accumulation of LPO products under conditions of 24-h ischemia.

The survival rate of rats with cerebral circulatory disorders depends on the compensatory response of neurons. However, the compensatory phase of reversible metabolic changes during hypoxia is short-lasting. Therefore, pharmacological drugs should be used for protection of the nervous tissue from hypoxia.

Previous experiments showed that the antiischemic effect of piracetam is associated with stimulation of aerobic and anaerobic glucose oxidation and inhibition of LPO in mitochondria [3]. Our results are consistent with published data that TAU-15 is a metabolic drug with cytoprotective activity [11,12].

We showed that 10% rats survive by the 14th day of the study (Fig. 1). Piracetam increased the survival rate of animals to 30% by the 14th day of ischemia. However, this drug had no effect on the mortality rate of rats over the first 6 day of study. The delayed effect of piracetam is probably associated with potentiation of AMPA glutamate receptors that increase the toxic influence of glutamate in the early stage of ischemia [14]. Moreover, the antiischemic effect of piracetam manifests under conditions of stable hemodynamics and increase in glucose supply to the brain.

TAU-15 increased animal lifetime to 40%. As differentiated from piracetam, TAU-15 decreased the mortality rate of rats starting from the 1st day of ischemia.

The median survival time of rats receiving piracetam and TAU-15 was 8 and 11 days, respectively (*vs*. 6 days in control animals). On days 1-14 of ischemia the relative effectiveness of TAU-15 was much higher compared to piracetam (Fig. 2).

The drugs stimulating metabolism and improving microcirculation are most potent neuroprotectors [1,4]. High relative effectiveness of TAU-15 is related to metabolic protection and positive effect on cerebral circulation.

Our findings show that TAU-15 has neuroprotective activity. Test drug normalizes energy metabolism, inhibits LPO, and reactivates AOS in the ischemic brain. TAU-15 decreases the mortality rate of animals with ischemic changes in cerebral circulation. TAU-15 is more potent than piracetam in producing the cerebroprotective effect. These data indicate that a new taurine derivative holds much

promise for metabolic protection of the brain during ischemia.

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