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Endogenous Intoxication and the Role of Antioxidants in Motion Activity Correction with Traumatic Brain Injury in Rat Model

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Abstract

The aim of this study was to assess the effectiveness of cytoflavin and mexicor in the posttraumatic period of traumatic brain injury (TBI).

Materials and Methods: The experiments were carried out on 60 white non pedigree female rats weighing 180-200 g, with 15 rats in each series. TBI was modeled by a free-falling weight drop of 100 g from a height of 80 cm on the parietal-occipital area of the head. Blood samples were taken from the sublingual vein in an amount of 2.0 ml on Days 1, 3, 7, and 12 after the alteration. After TBI, in Group 1, 15 rats received an intraperitoneal injection of 2-ethyl-6-methyl-3-hydroxypyridine succinate (mexicor) for 10 days in a daily dose of 8.0 mg/kg. In Group 2, 15 rats received an intraperitoneal injection of cytoflavin for 10 days in a daily dose of 0.2 ml/kg. The activity of lipid peroxidation and antioxidant protection system in the blood plasma was determined by a biochemicoluminescence method. Analysis of animal motion activity included the determination of the ability to balance and to stay at the wooden bar, time spent for moving on the bar from the bright light source to the darkroom, and paw slip frequency.

Results: Mexicor and cytoflavin decreased the level of oxidative processes in rat model with TBI and the development of secondary brain injury. The positive dynamics in restoring pro- and antioxidant system balance was combined with positive changes in motor function. (**International Journal of Biomedicine. 2019;9(1):61-65.**)

Key Words: traumatic brain injury • rats • lipid peroxidation • antioxidant protection system

Abbreviations

AOS, antioxidant protection system; $\mathbf{I_{max}}$, the maximum chemiluminescence intensity; **DC**, diene conjugate; **LPO**, lipid peroxidation; **LMMWS**, low and medium molecular-weight substances; **SB**, Schiff bases**; PSF**, paw slip frequency; **SBI**, secondary brain injury; **TAS**, total antioxidant status; **TBI**, traumatic brain injury; **TC**, triene conjugate.

Introduction

Traumatic brain injury (TBI) has a high incidence worldwide and is associated with significant morbidity and mortality. According to the Centers for Disease Control, the total combined rates for TBI-related emergency department visits, hospitalizations, and deaths increased in the 2001–2010 decade.(1) The development of body hypoxic state is one of the main factors of acute TBI. In hypoxic state, the energy deficit, to say more exactly ATP deficit, provokes homotypic metabolic and structural changes in various organs and tissues,(2) as well as LPO activation. In turn, LPO products aggravate the disturbance of membrane structure and functions. They play a key role in the beginning and development of brain edema, which is manifested in TBI and may be realized in the disturbance of motion and cognitive function.(3-5)

One of the most effective ways to prevent and treat brain traumatic and ischemic lesions is the use of antihypoxic drugs. These are remedies that weaken or nullify hypoxic disturbances. Mexicor and cytoflavin are two such remedies.(6-9) These

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medicines are largely used to manage ischemic conditions.[10-12]

The aim of this study was to assess the effectiveness of cytoflavin and mexicor in the posttraumatic period of TBI.

Materials and Methods

The experiments were carried out on 60 white non-pedigree female rats weighing 180-200g, with 15 rats in each series. All animals were given access to food and water *ad libitum*. Experiment was performed in accordance with the Order of the Ministry of Health of the Russian Federation №267 (19.06.2003) "On approval of the rules of Good Laboratory Practice» (GLP).

Experimental model of TBI

The animals were fixed on a plate, and blood was collected from a sublingual vein in an amount of 2.0 ml. It was 8%–9% of the circulating blood volume. TBI was modeled by a free-falling weight drop of 100g from a height of 80 cm on the parietal-occipital area of the head.[13] Blood samples were taken from the sublingual vein in an amount of 2.0 ml on Days 1, 3, 7, and 12 after the alteration. Such blood sampling technology simulates the fractional blood loss, which, during the 10 days of the posttraumatic period, was 32%–36% of the volume of circulating blood in the rats.

Treatment

After TBI, in Group 1, 15 rats received an intraperitoneal injection of 2-ethyl-6-methyl-3-hydroxypyridine succinate (mexicor) for 10 days in a daily dose of 8.0mg/kg (a solution for intravenous and intramuscular administration, JSC EkoFarmInvest, Russia). In Group 2, 15 rats received an intraperitoneal injection of cytoflavin for 10 days in a daily dose of 0.2 ml/kg (a solution for intravenous administration, OOO NTFF Polisan», Russia). In Group 3 (control group-CG), 15 rats received an intraperitoneal injection of physiological saline solution in the same volume. The administration of the drugs was started 1 hour after TBI. The values of the physiological norm of the studied parameters were determined in intact rats (n=15).

The activity of LPO and ASO in the blood plasma was determined by a biochemicoluminescence method[14] using the biochemiluminometer Lum-5773. The following indices of chemiluminogram were analyzed: total activity of free-radical oxidation (I_{max}) and TAS (tgα). LPO intensity was defined by the concentration of DC/TC of polyunsaturated fatty acids as well as SB by a spectrophotometry method.[15] using the RF-5301 PC, Shimadzu, (Japan). Each phase was analyzed in comparison with the respective group at the following wavelengths: 220 nm (isolated double bonds absorption), 232 nm (DC absorption), 278 nm (TC absorption), and 400 nm (SB absorption). The level of DC, TC, and SB was defined by E232/E220, E278/E220, E400/E220 and it was expressed in terms of relative units (RU).

The distribution index in relation to the content of middle molecules at wavelengths of 238 nm, 254 nm, 266 nm, and 282 nm was defined for assessment of LMMWS.[16] The calculation of the final result was made using the formula:
LMMWS=1.013×(8×E238+16×E254+44×E266/3+64×E282/3.

Animal motion activity analysis

The motor disorders were defined by the method of K.Saatman et al.[17] The ability to balance and to stay at the wooden bar, time spent for moving on the bar from the bright light source to the darkroom, and PSF were defined in the posttraumatic period.

The statistical analysis was performed using the statistical software Microsoft Excel. The mean (M) and standard error of the mean (SEM) were calculated. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney *U*-test. A probability value of $P<0.05$ was considered statistically significant.

Results

The research results showed that the endointoxication and LPO activation in blood plasma took place in the TBI groups of animals (Table 1).

Table 1.

Parameters of the endointoxication and LPO activation in the TBI posttraumatic period

Variable	Intact rats	Group	Period after TBI (day)			
			1	3	7	12
LMMW, RU	5.65±0.43	CG	9.09±0.55*	8.70±0.17*	8.78±0.33*	7.15±0.4*
		Group 1	7.69±0.74*	6.89±0.50^	6.84±0.37^	5.70±0.58
		Group 2	6.48±0.28^	6.86±0.86	5.79±0.49^	5.24±0.29^
I max, mV	1.64±0.14	CG	3.05±0.14*	2.74±0.16*	1.97±0.17	1.93±0.07
		Group 1	2.32±0.11*^	1.98±0.08*^	1.51±0.1^	1.27±0.012^
		Group 2	2.42±0.24*^	2.03±0.1*^	1.33±0.21^	1.36±0.24^
tga, mV/sec	3.86±0.06	CG	3.58±0.11*	3.56±0.18	3.41±0.23	3.31±0.15
		Group 1	6.69±0.51*^	4.87±0.31*^	4.33±0.42	4.88±0.07*^
		Group 2	6.43±0.38*^	4.91±0.31*^	4.59±0.19*^	4.17±0.08*^

**- P<0.05 between Group 1/Group 2 and a group of intact rats*
^- P<0.05 between Group 1/Group 2 and CG

Mexicor and cytoflavin induced a decrease in the intensity of the intoxication processes. This decrease manifested in a decrease of the plasma LMMWS level in all the stages of the TBI posttraumatic period. The findings showed that mexicor and cytoflavin supressed considerably the endointoxication in blood plasma and induced a decrease in the number of decay products. Besides, after the treatment with these medicaments, the exit of decay products to blood from injured tissues was less intensive.

Activation of free-radical processes and AOS depression were pronounced in the control group. Mexicor and cytoflavin restrained the free-radical oxidative stress. We found a decrease in I_{max} on Day 3 of the experiment by 28% in Group 1 and 26%, in Group 2 compared to the value of control gtoup. On Day 7, I_{max} in Groups 1 and 2 did not differ from that in the intact rats.

Mexicor and cytoflavin provoked positive changes in free-radical processes in the blood plasma (Table 2). Mexicor induced a decrease in the level of DC and TC on Day 3 of the experiment by 29% and 44% relative to CG, respectively. The correction effect of cytoflavin was weaker.

The levels of DG, TC and SB in Group 2 were normalized by Day 7 of the experiment.

We observed tonic and clonic seizures just after TBI for 2-4 sec. Animals lost sensitivity and they had been in a lateral position for 10-20 sec. The study of motor response showed a worsening of motor function up to the end of Day 1 of the experiment (Table 3).

The positive dynamics in restoring pro- and antioxidant system balance was combined with changes in motor function. Mexicor and cytoflavin injections induced the normalization of standing balance and walking ability. It manifested in a decrease in PSF as well as in time spent for moving on the bar. At the same time the mexicor effect was more strongly pronounced. PSF decreased by 55.6% and 44.4% by the end of Day 1 in Group 1 and Group 2, respectively. The positive dynamics in the motion activity indices was evident up to the end of Day 1 after the mexicor and cytoflavin injections. A significant improvement in motion reactions was registered on Day 3 of the experiment. The level of these indices achieved the intact animal value by Day 7 after the mexicor injection and by Day 12 after the cytoflavin injection.

Table 2.

Changes in the intensity of LPO in the blood plasma of rats in the posttraumatic period

Variable	Intact rats	Group	Period after TBI (day)			
			1	3	7	12
DC, RU	0.12±0.01	CG	0.22±0.02*	0.21±0.02*	0.16±0.02	0.12±0.02
		Group 1	0.16±0.01*^	0.15±0.01*^	0.13±0.02	0.09±0.01*
		Group 2	0.17±0.02*	0.17±0.03*	0.08±0.03^	0.08±0.04
TC, RU	0.08±0.01	CG	0.15±0.03*	0.16±0.03*	0.16±0.02*	0.08±0.02
		Group 1	0.12±0.02	0.09±0.01^	0.08±0.03^	0.07±0.01
		Croup 2	0.10±0.02	0.12±0.01*	0.09±0.02	0.06±0.03
SB, RU	8.43±0.84	CG	9.35±0.96	12.99±0.98*	11.06±0.92*	9.29±0.75
		Group 1	10.25±1.03	8.00±0.44^	8.03±1.33	8.25±0.41
		Group 2	9.89±0.89	10.94±0.84*	8.66±0.64^	8.90±0.87

**- P<0.05 between Group 1/Group 2 and a group of intact rats*
^- P<0.05 between Group 1/Group 2 and CG

Table 3.

Animal motion activity analysis

Variable	Intact rats	Group	Period after TBI (day)			
			1	3	7	12
Moving on bar, score	2.5±0.1	CG	6.8±0.9*	5.7±0.9*	4.9±0.5*	4.5±0.7*
		Group 1	4.2±0.8*^	4.0±0.4*	2.9±0.3^	2.6±0.4^
		Group 2	4.5±0.6*^	3.6±0.3*^	3.3±0.3*^	2.3±0.1^
PSF, numbers	1.0±0.1	CG	3.6±0.7*	2.8±0.6*	2.0±0.5	2.2±0.5*
		Group 1	1.6±0.7	1.3±0.4	1.1±0.2	0.8±0.2^
		Group 2	2.0±0.4*	1.7±0.3*	1.5±0.3	1.0±0.1^
Time spent for moving on the bar, score	1.5±0.1	CG	3.4±0.4*	2.9±0.3*	2.2±0.2*	2.0±0.4
		Group 1	1.8±0.2^	1.6±0.2^	1.4±0.2^	1.4±0.2
		Group 2	1.9±0.2^	1.8±0.1*^	1.8±0.2	1.4±0.1

**- P<0.05 between Group 1/Group 2 and a group of intact rats*
^- P<0.05 between Group 1/Group 2 and CG

Discussion

Our results showed that mexicor and cytoflavin decreased the level of oxidative processes with TBI and the development of secondary brain injury. Secondary brain injury represents the cascade of biochemical inflammatory stress reactions, which provoke brain ischemia and worsen considerably the severity of the general condition. These reactions also hinder the restoration of psychical and moving activity.[18] In addition, the processes of neuroregeneration and neuroprotection start at the same time as the secondary brain injury cascade. Specific intracellular neuroregulatory proteins and pluripotent stem cells, being neurotrophic factors, play a key role in these processes. However, in TBI (especially, in severe cases) the regeneration function of nerve tissue gives way to the processes of secondary brain injury because the site of primary brain injury is too large and the total body reactivity decreases; thus, the speed of the development of secondary brain injury is higher than that of the reparative processes.

The mexicor and cytoflavin injections in the posttraumatic period of TBI induced a decrease in endointoxication and oxidative stress by increasing the antioxidative blood potential in all stages of the experiment. These medications interrupted the formation of Schiff bases at the level of diene- and triene conjugates. Mexicor was the most effective in the first stage of the posttraumatic period (1-3 days after TBI). Probably, differences between effect manifestations are associated with differences in the composition of these "succinate" medications. Mexicor is a heteroaromatic antioxidant, in which the succinate is connected by covalent link with antioxidant emoxypine.[6] Cytoflavin is a complex preparation, the composition of which comprises the following components: succinate acid, riboflavin, riboxinum, and nicotinamide.[20] Earlier it was shown that this group of preparations decreases the intensity of LPO both in blood plasma and in cell membranes.[21]

It is very important to note that mexicor and cytoflavin promoted a decrease in the LMMWS content. The catabolic component of LMMWS is represented by low molecular weight compounds—protein breakdown products. The catabolic anabolic component of LMMWS is represented by a complex of peptide compounds—protein proteolysis products. Peptide compounds are similar by their structure to regulatory peptides, and they may provoke abnormality in the metabolism and function of cells. They may also provoke the appearance of drug resistance by blocking T-cell receptors. The study results showed that cytoflavin and mexicor restored the functioning of cell membranes due to antioxidant properties and decreasing LMMWS on the surface of the membranes. A decrease in LMMWS content is evidence of reduced inflammation.

Given that mexicor and cytoflavin are succinic acid drugs, it is possible to suppose that the restoration of metabolism in the cells involves restoring homeostasis. The restoration of movement function of the experimental animals proves this thesis. Thus, it can be concluded that mexicor and cytoflavin are multimodal drugs with neuroprotective, antioxidant and anti-inflammatory properties.

Conflict of Interest

The authors declare that they have no competing interests.

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