GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Mechanisms of Psychopharmacological Effects of Granulocytic Colony-Stimulating Factor during Severe Hypoxia

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Granulocytic colony-stimulating factor produced a strong therapeutic effect during encephalopathy of different genesis. The preparation improved the psychoneurological status and increased the number of neural precursors in the paraventricular area of cerebral hemispheres in animals.

Key Words: *hypoxia; encephalopathy; neural precursors; granulocytic colony-stimulating factor*

Nearly all diseases, traumas, and poisonings are accompanied by hypoxia and changes in the energy metabolism [3]. Inhibition of aerobic oxidation in the brain tissue reorganizes the central nervous system (CNS), modulates integrative and triggering activity of neurons, and leads to the formation of a qualitatively new pattern of the interaction between brain structures, while during decompensation of adaptive mechanisms it triggers a variety of pathological processes leading to progressive neurological disorders and cognitive dysfunction (encephalopathy) [1,4].

Recent studies of the properties and functional activity of multipotent precursor cells resulted in the development of a new approach to the therapy of various diseases — treatment with stem cells [6,7,9]. However, administration of undifferentiated or low purified cells into the organism is hazardous without reliable cell technologies [11,13]. Another approach is based on mobilization of endogenous stem cells with pharmacological agents (*e.g.*, granulocytic colony-stimulating factor, CSF) [8,10].

Here we studied the effect of granulocytic CSF on the psychoneurological status and number of neural precursors in the brain during severe hypoxia.

MATERIALS AND METHODS

Experiments were performed on 366 CBA/CaLac mice (class I conventional mouse strain) weighing 18-20 g and obtained from the nursery of the Department of Experimental Biomedical Modeling (Institute of Pharmacology, Tomsk Research Center). Hypoxic hypoxia and 2 regimens of hemic hypoxia served as the experimental models. Hypoxic hypoxia was modeled by placing the animal in a 500-ml sealed chamber (2 times with a 10-min interval). The mice were removed from the chamber after termination of generalized convulsions and/or respiratory arrest for 10-15 sec. Hemic hypoxia was modeled by intraperitoneal injection of phenylhydrazine hydrochloride in a single dose of 150 mg/kg or blood sampling from the retroorbital sinus through a graduated Pasteur pipette washed with hepa-

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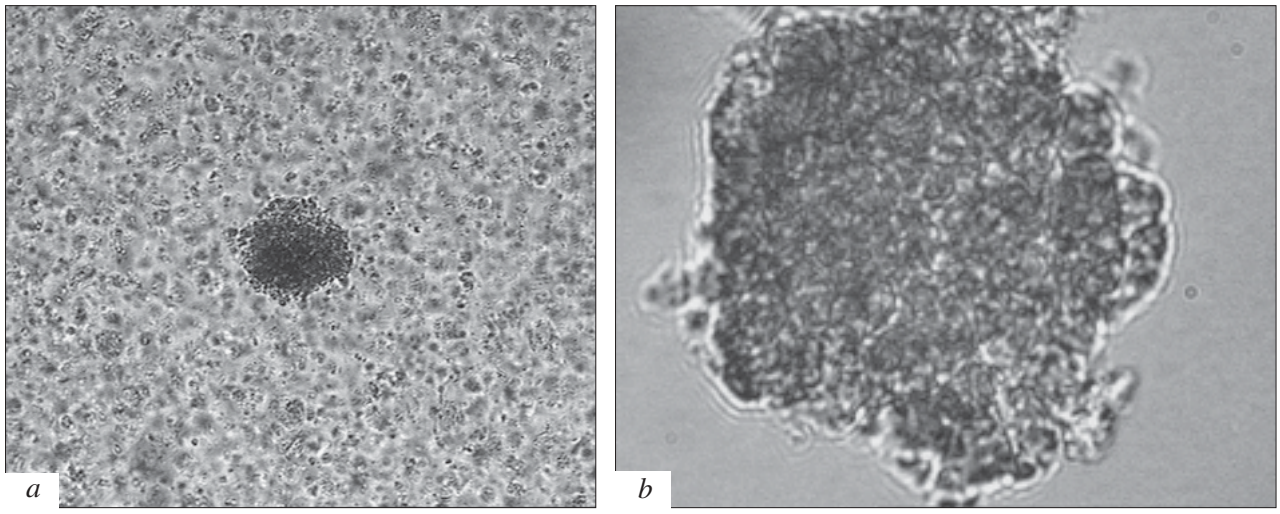

Fig. 1. Formation of neurospheres on day 7 of culturing (native preparation); ×300 (*a*), ×650 (*b*).

rin solution over 2-3 h (70% circulating blood volume, CBV, 3 procedures). The volume of withdrawn blood was estimated taking into account that CBV in rodents corresponds to $^1/_{13}$ of their body weight. Changes in the psychoneurological status were evaluated by reproduction of a conditioned passive avoidance response (days 7, 14, and 21) [2] and disturbances in orientation and exploratory activity in an open field (days 3, 7, 14, and 21) [2,14]. The preparation of recombinant human granulocytic CSF (rhG-CSF; Neipogen, Hoffman-la Roche) was dissolved in 0.2 ml solvent and injected subcutaneously in a daily dose of 125 μg/kg for 5 days immediately after hypoxia. Control animals received an equivalent volume of physiological saline. The number of neural precursors in the brain was estimated on days 1, 3, and 7. Nervous tissue was sampled from the paraventricular area of cerebral hemispheres using a BM-51-2 binocular microscope (×8.5). The sample (~3 mm^3) was immediately placed in 0.02% EDTA (Sigma) containing 1.25 g/liter trypsin (Sigma) and 6 g/liter glucose and incubated at 20-22°C for 10-15 min. Nervous tissue was dissociated to a suspension of nerve cells. The incubation medium was passed through a needle (diameter 2 mm) for 5-7 min. The suspension was filtered through a Capron mesh and washed 2 times by centrifugation at 1500 rpm for 5-10 min. The cells were placed in DMEM (Serve) containing 6 g/liter glucose, 280 mg/liter L-glutamine (Sigma), 50 mg/liter gentamicin (Serva), and 25 mg/liter porcine multipotent insulin (Novo Nordisk) and suspended. The total number of nucleated cells was estimated. Cell viability was determined using 0.1% trypan blue. The concentration of living cells in the same medium containing 25 ng/ml fibroblast growth factor (FGF-basic, Sigma) was brought to 10^5 cells/ml. The suspension (0.5 ml) was placed in 24-well plastic plates (Costar). The culture was incubated in a CO_2-incubator (Jouan) at 37°C, 5% CO_2, and 100% humidity for 7 days. Neurospheres (round structures containing more than 50 cells, Fig. 1) were counted after incubation using an MBS-9 binocular microscope (×56). The results were analyzed by methods of variational statistics (Student's *t* test, nonparametric Wilcoxon—Mann—Whitney *U* test).

RESULTS

Hypoxia of different genesis produced significant changes in the psychoneurological status of animals. We revealed a significant increase in horizontal activity and coefficient of movement asymmetry (Table 1). Total locomotor activity increased after hypoxic hypoxia (day 3) and blood loss (days 3, 7, 14, and 21). Conditioned passive avoidance performance was significantly impaired at all terms after hypoxia of different genesis (to 45.5, 0, and 11.1% on day 21 after hypoxia in a sealed chamber, administration of phenylhydrazine hydrochloride, and blood loss, respectively). Spontaneous mortality rate reached 27.3, 31.25, and 20% on day 21 after hypoxia in a sealed chamber, administration of phenylhydrazine hydrochloride, and blood loss, respectively.

The number of neural cells in the paraventricular area of cerebral hemispheres increased after hypoxia. These changes were significant 3 and 7 days after blood loss and administration of the hemolytic poison, respectively (Fig. 2). The number of stem cells in the nervous tissue decreased 1 day after hemic hypoxia (phenylhydrazine hydrochloride injection). It was probably related to cell damage with the toxic agent [5].

These data show that hypoxia of different genesis led to the development of encephalopathy manifesting in serious changes in the psychoneurological status.

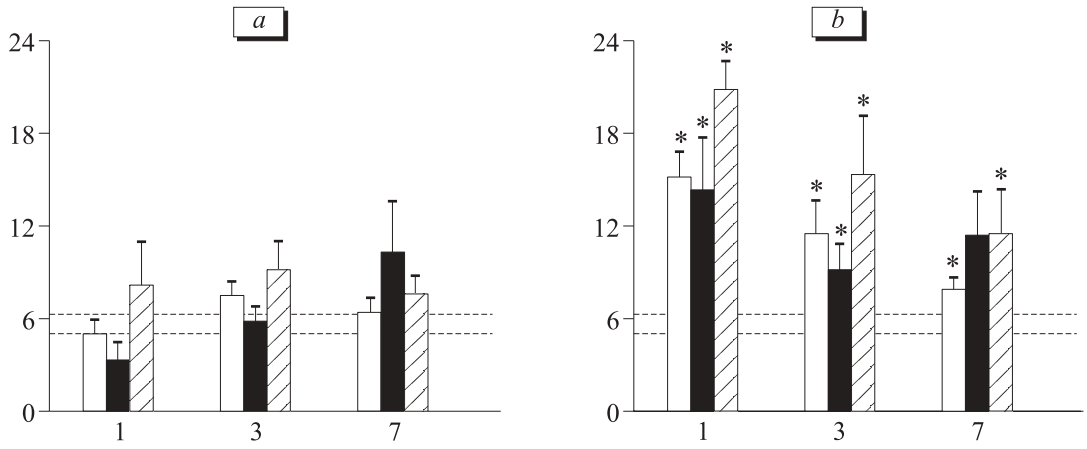

Fig. 2. Number of neural precursor cells in the paraventricular area of cerebral hemispheres in CBA/CaLac mice after hypoxia in sealed chamber (light bars), administration of phenylhydrazine hydrochloride (dark bars), and blood loss (shaded bars). Absence of preparation (*a*); course treatment with rhG-CSF immediately after hypoxia (*b*). Abscissa: days. Ordinate: value, per 10^5 karyocytes. **p*<0.05 compared to *a*. Area between dotted lines: confidence interval in intact mice (*p*<0.05).

Activation of mechanisms of "deep reserves" in the brain nervous tissue (stem cells) was insufficient.

We studied the possible psychopharmacological effect of rhG-CSF during hypoxia. The test preparation alleviated or completely abolished signs of brain dysfunction, which depended on the genesis of hypoxia. The course of treatment with rhG-CSF immediately after oxygen deficiency of different genesis prevented the development of memory disorders, which manifested in increased number of animals with normal response. Moreover, the preparation abolished changes in orientation and exploratory activity of mice in the open field (Table 1). The mortality rate in mice receiving rhG-CSF decreased to 10% on day 21 after hypoxia produced by phenylhydrazine administration.

The number of neural precursors in the brain increased after hypoxia in the sealed chamber, blood loss (days 1, 3, and 7), and administration of phenylhydrazine hydrochloride (days 1 and 3) compared to animals not receiving the preparation (Fig. 2).

Our results suggest that granulocytic CSF produces a strong therapeutic effect during encephalopathy of different genesis. The effect of this preparation is associated with a decrease in glutamate excito-

TABLE 1. Effect of rhG-CSF on Orientation and Exploratory Activity of CBA/CaLac Mice (arb. units, $X \pm m$)

Days		Coefficient of movement asymmetry			Horizontal locomotor activity		
		hypoxia in a sealed chamber	administration of phenyl-hydrazine hydrochloride	blood loss	hypoxia in a sealed chamber	administration of phenyl-hydrazine hydrochloride	blood loss
3rd	intact	0.49±0.06	0.47±0.03	0.28±0.04	8.00±1.74	10.44±2.22	3.90±0.77
	control	0.75±0.05*	0.60±0.04*	0.52±0.03*	26.43±4.97*	12.11±1.17	15.10±2.76*
	G-CSF	0.56±0.03$^+$	0.49±0.04	0.29±0.03$^+$	8.71±2.11$^+$	15.2±3.6	3.44±1.17$^+$
7th	intact	0.56±0.07	0.39±0.04	0.33±0.03	12.60±2.21	9.30±1.96	5.7±1.32
	control	0.75±0.03*	0.66±0.03*	0.60±0.03*	23.86±4.38*	19.75±3.77*	22.5±3.3*
	G-CSF	0.61±0.12	0.45±0.1$^+$	0.38±0.04$^+$	15.62±2.19	17.36±4.05	6.34±1.49$^+$
14th	intact	0.62±0.09	0.40±0.03	0.35±0.03	17.13±3.59	7.10±1.03	4.10±0.78
	control	0.80±0.02*	0.58±0.08*	0.67±0.05*	26.25±2.39*	7.75±2.02	16.00±3.01*
	G-CSF	0.69±0.10	0.47±0.06	0.37±0.04$^+$	20.94±4.97	7.60±2.28	5.20±1.32$^+$
21st	intact	0.46±0.04	0.43±0.03	0.45±0.02	12.7±2.2	9.60±1.55	10.20±1.33
	control	0.58±0.03*	0.66±0.05*	0.68±0.03*	20.25±3.89	21.00±3.38*	23.22±3.50*
	G-CSF	0.50±0.03	0.42±0.04$^+$	0.49±0.02$^+$	13.60±1.98	7.90±1.24$^+$	11.07±1.31$^+$

Note. *p*<0.05: *compared to intact animals; $^+$compared to the control.

toxicity [12] and mobilization of endogenous stem cells in the organism.

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