Effects of Cytoflavin and Neuronol on Morphological Changes in the Brain and Survival of Rats with Ischemic Disturbances in Cerebral Blood Flow

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Cytoflavin and neuronol produce vasoactive and neuroprotective effects in rats with cerebral ischemia. Vasoactive activity of neuronol was higher than that of cytoflavin. These differences were most pronounced at the level of microcirculation. Test preparations were equally potent in producing the neuroprotective effect. Cytoflavin and neuronol markedly decreased the mortality rate of animals. Over the first 6 h of ischemia the relative effectiveness of cytoflavin was higher than that of neuronol. However, neuronol exceeded cytoflavin in the relative effectiveness during the follow-up period (days 1-21).

Key Words: *cerebral ischemia; morphological changes; survival rate; relative effectiveness; cytoflavin; neuronol*

Hemodynamic disturbances, hypoxia, oxidative stress, and glutamate excitotoxicity play a role in the pathogenesis of cerebral ischemia [1,4]. The therapy of this disease should involve vasoactive and neuroprotective compounds capable of modulating various stages of the ischemic cascade.

The complex preparation cytoflavin was synthesized at the Scientific Technological Pharmaceutical Company Polysan. It consists of succinic acid, riboxine, nicotinamide, and riboflavin mononucleotide. Previous studies showed that cytoflavin possesses antihypoxic and antioxidant properties and produces a cerebroprotective effect during postischemic reperfusion brain injury [2]. A new pharmaceutical preparation neuronol synthesized at the Scientific Technological Pharmaceutical Company Polysan contains piracetam and pyridoxine-HCl.

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Here we compared the effects of cytoflavin and neuronol on the vascular system, brain tissue, and survival of rats with ischemic disturbances in cerebral blood flow.

MATERIALS AND METHODS

Experiments were performed on 150 male outbred albino rats weighing 180-200 g. The animals were tested in the open-field. Further experiments were performed with intermediate-activity rats. Cerebral ischemia was modeled by ligation of the left common carotid artery and reduction of blood flow in the right common carotid artery (by 50% of baseline) [8]. The animals not receiving injections served as the control (ischemia). Other rats were exposed to ischemia and additionally received cytoflavin or neuronol.

Neutralized solutions of cytoflavin and neuronol (175 and 125 mg/kg, respectively) and physiological saline were injected intraperitoneally 30 min and 1 day after occlusion due to bad postoperation state of animals. In the follow-up period (days 3-21) the test preparations were given perorally once a day with starch suspension.

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We estimated the survival rate and median survival rate (time-to-survival for 50% rats). The relative effectiveness of preparations was calculated as follows:

$$Z = \frac{P\text{-}C}{1\text{-}C},$$

where Z is relative effectiveness of preparations; P is the percent of survivors after treatment with test preparations; and C is the percent of survivors in the control [5].

For morphological examination sagittal sections of the left and right hemispheres were prepared. The state of the vascular system was determined by staining with hematoxylin and eosin. Nissl staining evaluated the state of neurons, glia, and conducting pathways in various brain regions. We presented photos of histological sections of left hemisphere tissue with most severe injury.

RESULTS

Spasm, rupture, and plasma impregnation of the vascular wall were found in muscle and elastic arteries rats on days 3-7 of cerebral ischemia. We revealed perivascular plasmorrhages and hemorrhages in brain membranes (Fig. 1, *a*). Arterioles and capillaries of the intramural vascular system were characterized by spasm or dystonia, proliferation of the endothelium, and vasoconstriction. These changes were accompanied by ve-

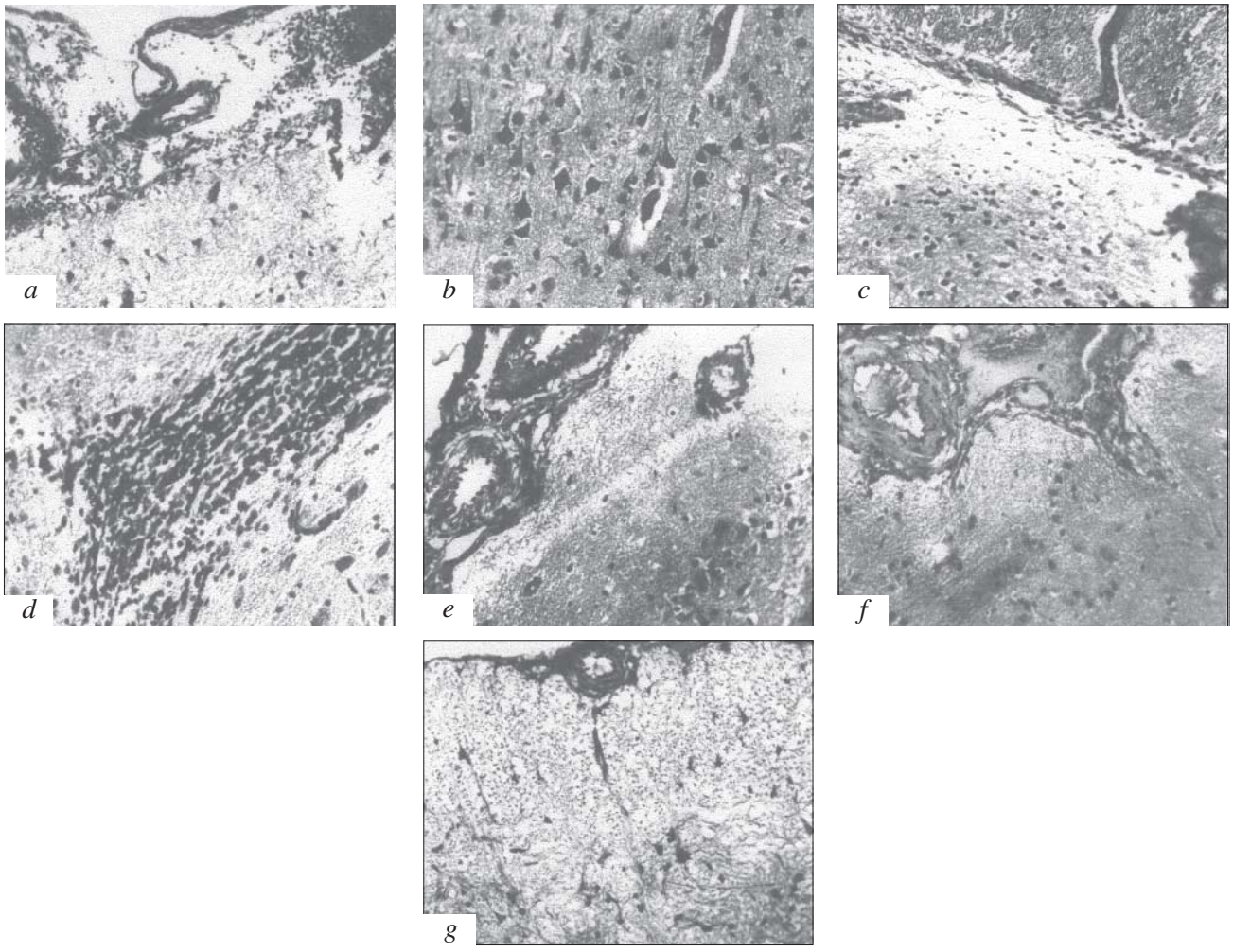

Fig. 1. Effects of cytoflavin and neuronol on morphological signs of ischemic brain injury in rats. Rupture, spasm, and plasma-driven permeation of the arterial wall, hemorrhage in the membrane and marginal brain region in the control and on day 3 of ischemia (*a*). Arterialization of capillaries and severe diffuse injury of cells in the cerebral cortex in the control and on day 14 of ischemia (*b*). Sclerosis of membranes and vessels, arterialization of capillaries, swelling and extended demyelination of conducting pathways in the control and on day 21 of ischemia (*c*). Replacement macrofocal gliosis in the control and on day 21 of ischemia (*d*). Absence of progressive arteriosclerosis, ischemic injury of neurons, and demyelination of conducting pathways on day 21 of ischemia; treatment with neuronol (*e*). Arteriosclerosis without narrowing and deformation of the lumen and perivascular sclerosis, focus of demyelination, absence of gliosis in the ischemic focus on day 21 of ischemia; treatment with cytoflavin (*f*). Hyalinosis of individual arterioles, absence of gliosis, microfocal demyelination on day 21 of ischemia; treatment with cytoflavin (*g*). Staining with hematoxylin and eosin (×140, *a*, *d-g*). Nissl staining (×140, *b*, *c*).

nous plethora and appearance of small perivascular hemorrhages. The progressive pathological process and vascular sclerosis were detected in the late postischemic stage (days 14-21, Fig. 1, *b*, *c*). The lumen of some large arteries was obliterated. We found hyalinosis of arterioles and arterialization of capillaries (widening and wall thickening).

Ischemic brain was characterized by severe damage to neurons: pronounced homogenization and basophilia of the cytoplasm, fusion of nuclei with pyknotic cytoplasm, and appearance of uneven cells encrusted with the granular material (Fig. 1, *b*). We found fields of sharply swollen cells, edematous necrobiosis, neuronophagy, death of groups or fields of neurons, and replacement gliosis at the site of dead cells (Fig. 1, *d*). Extended injury in conducting pathways was accompanied by demyelination (Fig. 1, *c*). These pathological changes were revealed in the cerebral cortex, medulla oblongata, subcortical nuclei, hypothalamus, Purkinje cells, and dentate cerebellar nucleus. Neurodegenerative changes in the hippocampus were less pronounced.

Cytoflavin and neuronol improved the state of the vascular system in the ischemic brain. The test preparations reduced spasm and decreased permeability of arteries on day 3 of ischemia. Cytoflavin and neuronol reduced the incidence and area of perivascular plasmorrhages and hemorrhages. In the late postischemic stage (days 14-21) these preparations markedly decreased or completely abolished progressive arteriosclerosis, hyalinosis of arterioles, and arterialization of capillaries (Fig. 1, *e*-*g*). Cytoflavin and neuronol increased the density of capillaries. We compared the effects of preparations on the vascular system. Vasoactive activity of neuronol was higher than that of cytoflavin. These differences were most pronounced in the microcirculatory bed.

Cytoflavin and neuronol produced a positive effect on ischemic brain tissue. They decreased the severity of ischemic injury. Swelling of neurons, edematous necrolysis, and neuronophagy of cells were rarely found (Fig. 1, *e*). We found only microfocal replacement gliosis (Fig. 1, *g*). The test preparations protected conducting pathways and decreased the severity and area of demyelination (Fig. 1, *e*-*g*). No differences were revealed in the effect of cytoflavin and neuronol on the state of brain tissue.

Cytoflavin and neuronol decreased the mortality rate of rats with impaired cerebral blood flow. It should be emphasized that neuronol was more potent than cytoflavin (Fig. 2). On day 21 of ischemia the survival rate of animals receiving neuronol and cytoflavin was 54.5 and 45.2%, respectively (*vs*. 32.2% in the control). The median survival time of animals treated with neuronol and cytoflavin surpassed 21 and 9 days, respectively (Fig. 2). On days 1-21 of ischemia the relative effectiveness of neuronol was 1.4-2.8 times higher than that of cytoflavin (Fig. 3).

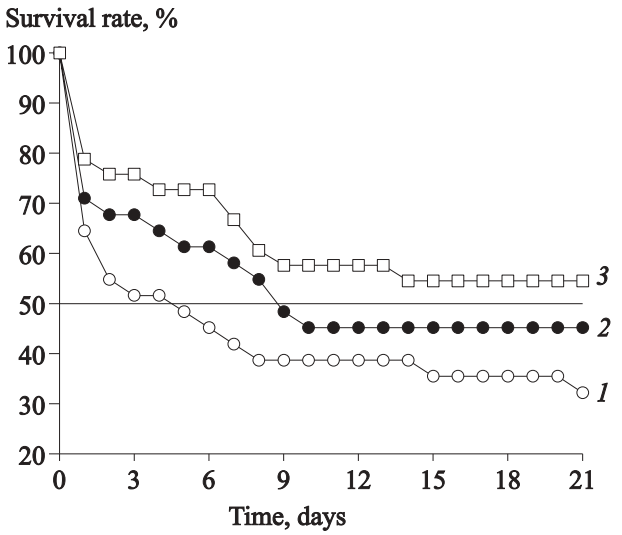

Fig. 2. Survival rate of rats with cerebral ischemia receiving cytoflavin and neuronol. Solid horizontal line: median survival time. Control (*n*=31, *1*), cytoflavin (*n*=31, *2*), neuronol (*n*=33, *3*).

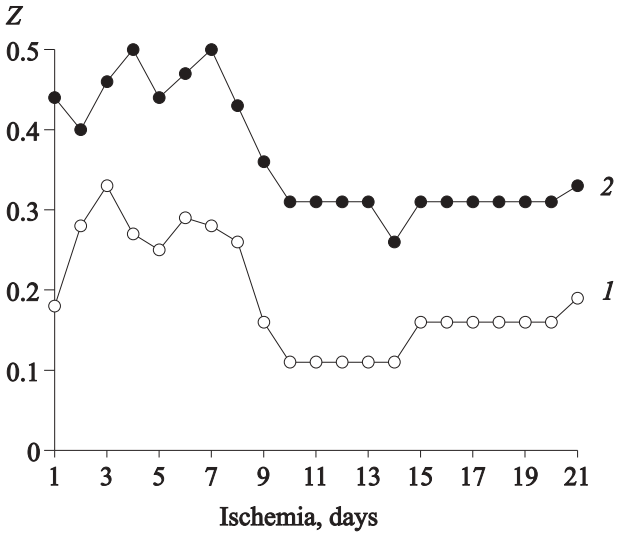

Fig. 3. Relative effectiveness (Z) of cytoflavin (*1*) and neuronol (*2*) in rats with cerebral ischemia.

Hypoxia accompanies disturbances in cerebral blood flow and affects energy metabolism, which plays a major role in the maintenance of cell homeostasis [4, 10]. Cytoflavin and neuronol contain succinic acid and nicotinamide that activate succinate dehydrogenase- and NAD-dependent oxidative pathways in the mitochondrial respiratory chain [2,6,7]. The test preparations probably improved energy supply to cells. Morphological study showed that cytoflavin and neuronol are equally potent in producing the neuroprotective effect. However, vasoactive activity of neuronol was higher than that of cytoflavin. It probably contributes

to a greater effectiveness of neuronol on days 1-21 of ischemia.

Particular attention was given to relative effectiveness of cytoflavin and neuronol over the first 6 h after the incidence of ischemia ("therapeutic window"). During this period the relative effectiveness of cytoflavin was 1.7-fold higher than that of neuronol (0.65 and 0.38, respectively).

Glutamate excitotoxicity is maximum over the first 3-6 h after impairment of cerebral blood flow [4]. Published data show that piracetam potentiates AMPA glutamate receptor and increases the release of glutamate [9]. It can be hypothesized that piracetam counteracts the cerebroprotective effect of neuronol during the acute stage of ischemia. The decrease in glutamate excitotoxicity is accompanied by stabilization of hemodynamics and increase in the influx of glucose into the brain. During this period, neuronol becomes more effective than cytoflavin. Piracetam activates anaerobic and aerobic oxidation of glucose, which increases the energy potential of neurons [3].

Our results suggest that cytoflavin and neuronol produce vasoactive and neuroprotective effects and decrease the mortality rate in animals with cerebral blood flow disturbances. These data indicate that the test preparations hold much promise for the therapy of ischemic disturbances in cerebral blood flow. It is important that cytoflavin should be used in the acute stage of ischemia, while neuronol is most effective during the follow-up period.

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