# Neuroprotective Effect of Cytoflavin during Compression Injury of the Spinal Cord

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Cytoflavin normalized energy metabolism, decreased the intensity of lipid peroxidation, and reactivated the antioxidant system in the spinal cord of rats with compression injury at the level of $Th_{10}$-$Th_{11}$. The neuroprotective effect of the test preparation manifested in normalization of hindlimb motor function and decrease in mortality rate of animals with spinal cord injury. Neuroprotective activity of cytoflavin was higher than that of Cerebrolysin.

**Key Words:** *compression injury; spinal cord; ischemia; cytoflavin; Cerebrolysin*

Ischemia plays an important role in the pathogenesis of spinal cord injury. Ischemia resulting from hemodynamic disturbances triggers cascade pathobiochemical reactions (impairment of energy metabolism, intensification of free radical oxidation, development of oxidative stress and glutamate excitotoxicity, and accumulation of cytoplasmic $Ca^{2+}$). These changes are followed by secondary necrosis of the primarily damaged neurons [1,5,6].

Combination therapy of spinal cord injury should include preparations capable of preventing secondary ischemic injury in nerve cells. Cytoflavin (Polysan Research and Technological Pharmaceutical Company) exhibits antihypoxic and antioxidant properties and holds much promise in this respect. Previous studies revealed a therapeutic effect of cytoflavin during postischemic reperfusion injury of the brain and craniocerebral trauma [2,3].

Here we studied the effects of cytoflavin on complications of spinal cord compression. Cerebrolysin served as the reference preparation.

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## MATERIALS AND METHODS

Experiments were performed on 200 male outbred albino rats weighing 180-200 g. Laminectomy ($Th_{10}$-$Th_{11}$) was performed under ether anesthesia; the dura mater was not damaged. Compression of these segments in the spinal cord was produced using a metal rod (length 7 cm, diameter 3 mm) fixed in a universal stereotactic device. The rod was inserted to a depth of 5 mm from the level of the dura mater. The duration of compression was 1 min. Some animals were sham-operated.

The animals with spinal cord injury received physiological saline (control), cytoflavin (1.5 ml/kg), or Cerebrolysin (1 ml/kg). The test preparations were injected intraperitoneally. The first injection was made 30 min after injury. In the follow-up period cytoflavin and Cerebrolysin were administered 2 and 1 times a day, respectively. The therapeutic effect was assayed 1, 3, 7, and 14 days after injury.

The degree of hindlimb motor dysfunction was estimated by a 4-point scale: 1, normal; 2, moderate paraparesis; 3, severe paraparesis; 4, paraplegia [8]. We estimated survival rate and median survival rate (time corresponding to survival of 50% rats). The relative effectiveness of the test preparations was calculated as follows: $Z=(P-C)/(1-C)$, where $Z$ is the relative effectiveness of the preparation; $P$ is the ratio of sur-

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vived animals in the treatment group; and *C* is the ratio of survived animals in the control group.

The concentrations of lactic acid (LA), pyruvic acid (PA), malonic dialdehyde (MDA), and reduced glutathione (RG) and activity of superoxide dismutase (SOD) were measured in the liquor. Each sample contained the liquor from 3 rats.

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

## RESULTS

The rats with compression injury of the spinal cord had hindlimb paraplegia (4 points, Fig. 1). Cerebrolysin and cytoflavin decreased the severity of hindlimb motor dysfunction (Fig. 1).

Complications of spinal cord compression (functional and trophic changes in pelvic organs, conduction disorders for various types of sensitivity) caused animal death. The survival rate 6 days after injury was 94-92%, after 7-8 days 16-11% and on day 9 after injury all animals died (Fig. 2).

Cerebrolysin significantly decreased the mortality rate of rats with spinal cord injury. The survival rate over the first 7 days and on days 8-14 after injury was 100-59 and 53-41%, respectively. Cytoflavin was more potent than Cerebrolysin in increasing the survival rate. The survival rate in animals receiving cytoflavin was 100-87 and 84-52% over the 1st and 2nd weeks after injury, respectively (Fig. 2).

Cytoflavin more significantly improved the median survival rate than Cerebrolysin (Fig. 2). Control animals and rats receiving Cerebrolysin and cytoflavin survived over 5.5, 9.5, and 14 days, respectively.

The relative effectiveness of the test preparations was similar over the first 3 days after spinal cord injury. In the follow-up period the relative effectiveness of cytoflavin was 1.3-13 times higher than that of Cerebrolysin (Fig. 3).

LA concentration in the liquor increased to 333%, while PA content decreased to 31% on day 1 after compression injury of the spinal cord (Table 1). The LA/PA ratio reflecting the intensity of glycolytic or aerobic conversion of carbohydrates increased from 9.0 to 95.5 (by 10.6 times). These data illustrate inhibition of aerobic mechanisms of energy formation and stimulation of glycolysis.

The intensity of glycolysis decreased in the delayed period after spinal cord injury (days 3 and 7). It was confirmed by the decrease in the LA/PA ratio to 51.2 and 29.8, respectively. However, the intensity of glycolysis in treated rats was much higher than in sham-operated animals.

MDA concentration increased by 78%, while SOD activity and RG content decreased by 61 and 64%,

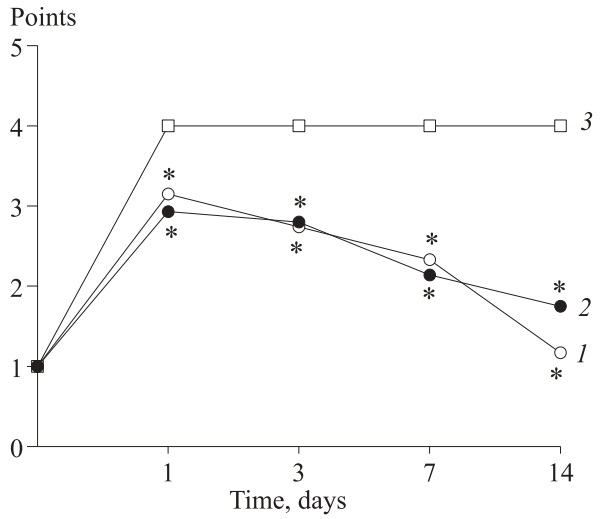


**Fig. 1.** Recovery of hindlimb motor function in rats with compression injury of the spinal cord: treatment with cytoflavin (*1*) and Cerebrolysin (*2*); control (*3*). *$p<0.001$ compared to the control.

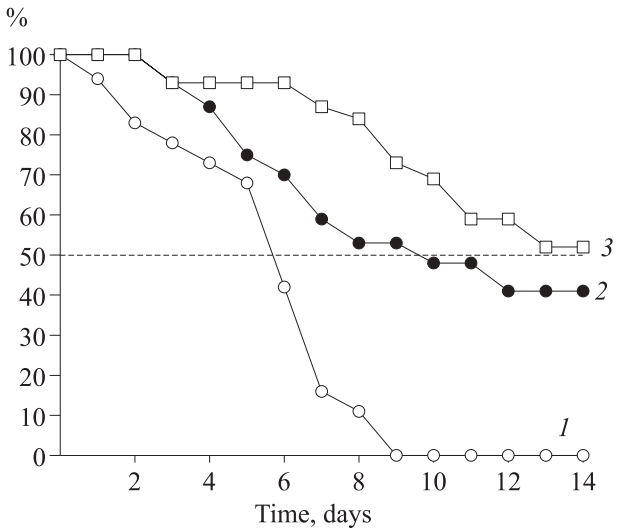


**Fig. 2.** Survival of rats with compression injury of the spinal cord: control (*1*); treatment with Cerebrolysin (*2*) and cytoflavin (*3*). Dotted line: median survival rate.

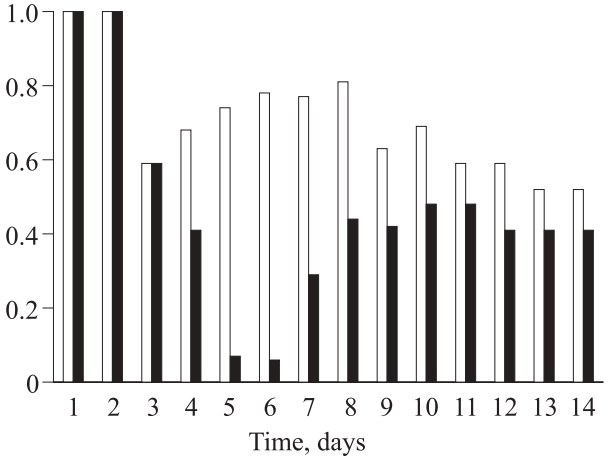


**Fig. 3.** Relative effectiveness of cytoflavin (light bars) and Cerebrolysin (dark bars).

**TABLE 1.** Effects of Cytoflavin and Cerebrolysin on Metabolic Parameters of the Liquor in Rats with Compression Injury to Spinal Cord (*M*±*m*, *n*=6-7)

| Group | | LA, μmol/g | PA, μmol/g | LA/PA | MDA, nmol/g | SOD, U/mg protein | RG, μmol/g |
|---|---|---|---|---|---|---|---|
| Sham-operation | | 1.72±0.03 | 0.19±0.01 | 9.0 | 4.19±0.05 | 0.56±0.03 | 1.32±0.08 |
| Injury, day 1 | placebo | 5.73±0.08* | 0.06±0.01* | 95.5 | 7.47±0.17* | 0.22±0.02* | 0.48±0.13* |
| | cytoflavin | 3.98±0.08+ | 0.07±0.01 | 56.9 | 5.81±0.14+ | 0.33±0.02+ | 0.53±0.03 |
| | Cerebrolysin | 4.92±0.12+ | 0.06±0.01 | 82.0 | 6.61±0.14+ | 0.31±0.02+ | 0.51±0.02 |
| Injury, day 3 | placebo | 4.61±0.21* | 0.09±0.01* | 51.2 | 5.82±0.10* | 0.31±0.03* | 0.63±0.04* |
| | cytoflavin | 3.10±0.17+ | 0.11±0.01 | 28.2 | 4.35±0.20+ | 0.38±0.02 | 0.71±0.04 |
| | Cerebrolysin | 3.54±0.12+ | 0.10±0.01 | 35.4 | 4.97±0.19+ | 0.36±0.01 | 0.65±0.04 |
| Injury, day 7 | placebo | 3.88±0.12* | 0.13±0.02* | 29.8 | 5.16±0.19* | 0.46±0.03* | 0.80±0.04* |
| | cytoflavin | 2.23±0.08+ | 0.13±0.01+ | 17.1 | 4.31±0.07+ | 0.46±0.02 | 0.96±0.02+ |
| | Cerebrolysin | 2.46±0.07+ | 0.11±0.01 | 22.4 | 4.61±0.19+ | 0.41±0.02 | 0.89±0.02 |
| Injury, day 14 | placebo | Died | Died | Died | Died | Died | Died |
| | cytoflavin | 2.12±0.19 | 0.15±0.01 | 14.3 | 4.22±0.07 | 0.51±0.02 | 1.10±0.04 |
| | Cerebrolysin | 2.09±0.10 | 0.13±0.01 | 16.1 | 4.41±0.13 | 0.49±0.02 | 1.20±0.02 |

**Note.** $p<0.05$: *compared to sham-operation; +compared to placebo.

respectively, on day 1 after spinal cord injury (compared to sham operation, Table 1). These changes illustrate hyperactivation of lipid peroxidation (LPO) and inhibition of the antioxidant system.

MDA concentration decreased, but did not return to the baseline level in the delayed period after injury (days 3 and 7). During this period activity of the antioxidant system increased, but was much lower compared to sham-operated rats.

The nootropic preparation Cerebrolysin contains active amino acids and peptides [9]. This preparation decreased the intensity of glycolysis and stimulated the aerobic pathway for energy formation (days 1-14, Table 1). However, the intensity of glycolysis remained above the baseline level on day 14 of therapy.

Cytoflavin contains succinic acid, riboxine, nicotine amide, and riboflavin mononucleotide [2]. Cytoflavin was more potent than Cerebrolysin in stimulating aerobic oxidation of substrates (Table 1). The LA/PA ratio in rats of the cytoflavin group was much lower compared to animals receiving Cerebrolysin.

Both preparations decreased the intensity of LPO and reactivated the antioxidant system. However, cytoflavin more significantly suppressed LPO than Cerebrolysin (days 1-7 of therapy). The intensity of LPO decreased, while activity of the antioxidant system returned to normal on day 14 of treatment with these preparations.

Our results suggest that cytoflavin produces a potent neuroprotective effect during compression injury of the spinal cord. This preparation normalizes energy metabolism, inhibits LPO, and reactivates the antioxidant system. The neuroprotective effect of cytoflavin manifests in normalization of motor function in the hindlimbs and decrease in the mortality rate.

Neuroprotective activity of cytoflavin is higher than that of Cerebrolysin. Cytoflavin not only normalizes metabolic processes in the nervous tissue, but also produces a vasoactive effect. This preparation decreases vascular permeability, abolishes progressive sclerotic reconstruction of vessels, and increases the density of capillaries during experimental cerebral ischemia [3]. Cytoflavin improves cerebral microcirculation in patients with after stroke ischemic disturbances in cerebral circulation [7].

Cytoflavin is a promising neuroprotector, which can be used in combination therapy of spinal cord compression.

## REFERENCES

1. I. A. Borshchenko, A. V. Baskov, A. G. Korshunov, and F. S. Satanova, *Vopr. Neirokhir*., No. 2, 28-31 (2000).
2. V. V. Bul'on, I. V. Zarubina, A. L. Kovalenko, *et al*., *Eksp. Klin. Farmakol*., **66**, No. 6, 56-58 (2003).
3. V. V. Bul'on, I. B. Krylova, N. R. Evdokimova, *et al*., *Byull. Eksp. Biol. Med.*, **139**, No. 4, 467-470 (2004).
4. V. V. Bul'on, L. K. Khnychenko, N. S. Sapronov, *et al*., *Ibid*., **129**, No. 2, 149-151 (2000).
5. E. I. Gusev and V. I. Skvortsova, *Cerebral Ischemia* [in Russian], Moscow (2001).
6. O. V. Povarova, E. I. Kalenikova, E. I. Gorodetskaya, and O. S. Medvedev, *Eksp. Klin. Farmakol*., **66**, No. 3, 69-73 (2003).
7. A. A. Skoromets, V. V. Nikitina, K. V. Golikov, *et al*., *Med. Akad. Zh*., **3**, No. 2, 90-97 (2003).
8. G. Z. Sufianova, L. A. Usov, A. A. Sufianov, *et al*., *Eksp. Klin. Farmakol*., **65**, No. 5, 58-61 (2002).
9. M. Windisch, *Brain Mechanisms and Psychotropic Drugs*, Eds. A. Baskys, *et al*., New York (1996), pp. 239-257.