PHARMACOLOGY AND TOXICOLOGY

Neuroprotective Effect of Afobazole on Rats with Bilateral Local Photothrombosis of Vessels in the Prefrontal Cortex

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We studied the neuroprotective effect of a new selective anxiolytic afobazole on rats with bilateral focal ischemic stroke in the prefrontal cortex caused by photothrombosis. Intraperitoneal injection of 5 mg/kg afobazole 1 h after surgery and over the next 8 days (daily treatment) produced a neuroprotective effect. Afobazole was far superior to the reference cerebroprotective drug cavinton (4 mg/kg) by neuroprotective activity.

Key Words: *afobazole; cavinton; neuroprotection; photothrombosis of the prefrontal cortex*

An original selective anxiolytic afobazole was synthesized at the V. V. Zakusov Institute of Pharmacology. Afobazole is listed in the Nomenclature of Pharmaceutical Preparations of Russia (registration certificate LS 000861 03.11.05).

Previous studies showed that afobazole has antioxidant properties, prevents overproduction of nitric oxide (NO), and increases superoxide dismutase activity during experimental cerebral ischemia [1,12]. Afobazole prevents the increase in activity of neuronal NO synthase and, therefore, inhibits the NO synthase pathway of NO production in rat brain neurons. This effect of afobazole is accompanied by a decrease in anxiety of animals with local cerebral ischemia [1].

In vitro experiments with cultured HT-22 cells (immortalized mouse hippocampal cells) showed that afobazole produces a neuroprotective effect under conditions of oxidative stress and glutamate toxicity [4]. Direct studies on the model of hemorrhagic stroke showed that delayed treatment with afobazole induces a protective effect [3]. Administration of afobazole before and after local ischemia caused by middle cerebral artery ligation is followed by reduction of pathomorphological changes in the perifocal area. The cerebroprotective effect of afobazole during cerebral ischemia is realized via activation of energy metabolism due to recovery of succinate dehydrogenase activity. It should be emphasized that activity of this enzyme decreases under conditions of cerebral ischemia [1].

Previous studies showed that afobazole improves cerebral blood flow and increases survival rate in rats with global intermittent ischemia caused by carotid artery ligation [8].

Photothrombosis of the prefrontal cortex in rats is accompanied by local ischemic injury, which extends over the width of the cortex [6,10]. Experimental photothrombosis results in local ischemic injury of the desired location, constant volume, and high reproducibility. This approach is required to perform a quantitative study of brain damage and

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to compare the effectiveness of various pharmaceutical preparations.

Here we studied the neuroprotective effect of afobazole on experimental rats with ischemic stroke caused by bilateral photothrombosis of vessels in the prefrontal cortex. We compared the effectiveness of afobazole and reference cerebroprotective drug cavinton.

MATERIALS AND METHODS

Experiments were performed on outbred male rats weighing 200-250 g. The animals were maintained in a vivarium under natural light/dark conditions and had free access to food and water.

The rats were intraperitoneally anesthetized with 3% chloral hydrate in a dose of 300 mg/kg. Animal's head was fixed in a Medikor stereotaxis to induce bilateral photochemical damage to the prefrontal cortex. Longitudinal midline skin incision was made with a scalpel. Skin flaps were moved apart. The periosteum was separated. A photosensitized staining agent Bengal rose (3% solution, 40 mg/kg) was injected into the jugular vein of anesthetized animals before surgery.

Photothrombosis was induced using an original device. Light beam (560 nm) was delivered from a halogen lamp (25 V, 250 W) to the intact cranial surface using a fiber-optic light guide (aperture slot 3 mm). This guide was placed at a distance of 1 mm from the cranial surface, 2.5 mm rostral to bregma, and 1.5 mm lateral to the sagittal suture (fields Fr1 and Fr2) [11]. The period of light exposure for each hemisphere was 20 min. The reaction between the fluorescent staining agent Bengal rose and light beam led to the release of free oxygen, which damages the vascular endothelium, platelet adhesion and aggregation, thrombus formation, and impairment of local blood flow. The lamp was cooled with a ventilator of the device to prevent thermocoagulation. Core body temperature in animals was maintained at 37.0-37.5°C (heating under an infrared lamp). Sham-operated rats were subjected to the same manipulations except for administration of Bengal rose.

Locomotor activity of rats was studied in a RODEO-1 automated open field to evaluate the homology of experimental groups. The number of vertical rearing postures and horizontal locomotor activity of animals were studied for 5 min. Experimental groups of animals were identical by locomotor activity in the open field (Table 1).

Experimental animals were divided into 4 groups: group 1, sham operation and administration of physiological saline; group 2, photothrombosis of the prefrontal cortex and administration of physiological saline; group 3, photothrombosis of the prefrontal cortex and administration of afobazole in a daily dose of 5 mg/kg; and group 4, photothrombosis of the prefrontal cortex and administration of cavinton in a daily dose of 4 mg/kg. The test drugs were injected intraperitoneally 1 h after photothrombosis and over the next 8 days (daily treatment). Control groups of ischemic and sham-operated animals received an equivalent volume of physiological saline. The cerebroprotective drug cavinton served as a reference preparation. Cavinton is extensively used in the therapy of patients with cerebrovascular disorders [5].

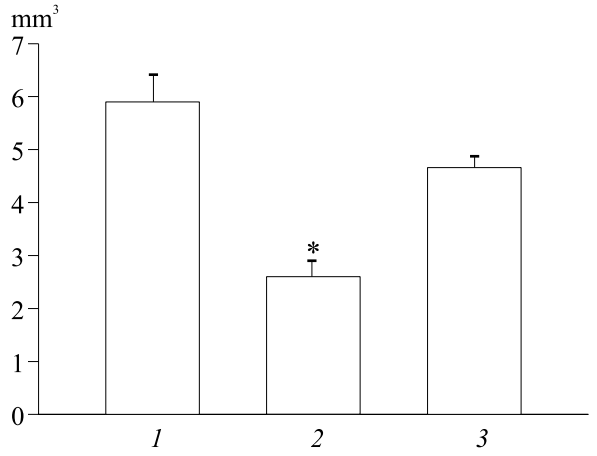

Fig. 1. Volume of focal ischemia on day 9 after photothrombosis of the prefrontal cortex in rat brain. Groups 2 (*1*), 3 (*2*), and 4 (*3*). *$p<0.05$ compared to animals of groups 2 and 4.

The volume of ischemic injury in the cerebral cortex was calculated as the product of the area of cortical damage and width of the cortex [9].

RESULTS

Bilateral ischemic injury in the frontal cortex of rats was followed by the development of focal ischemia. The ischemic zone extended over the width of the cortex and was separated from surrounding tissues by a well-defined boundary [6].

Afobazole was much more potent than cavinton in reducing the volume of ischemic injury in

TABLE 1. Initial Behavioral Parameters of Rats in the Open Field

Group	Number of vertical rearing postures	Horizontal activity
1 (*n*=10)	15.8±0.7	201.4±10.9
2 (*n*=7)	17.8±0.3	202.0±12.0
3 (*n*=13)	16.4±0.2	209.8±7.2
4 (*n*=7)	15.3±0.5	203±10

animals with bilateral photochemical damage to the prefrontal cortex of the brain ($p<0.05$, Fig. 1).

We conclude that afobazole produced a potent neuroprotective effect and reduced the volume of the ischemic zone in rats with bilateral ischemic injury in the prefrontal cortex due to photothrombosis. Our results are consistent with published data that afobazole induces a potent neuroprotective effect during ischemia of the cerebral cortex [3,7, 8]. Moreover, afobazole far surpassed the reference drug cavinton by neuroprotective activity.

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