Neuroprotective Properties of Afobazol *in Vitro*

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The effects of a novel selective anxiolytic afobazol on survival of HT-22 neurons were studied in the model of oxidative stress and glutamate toxicity. In both models, the neuroprotective effect of afobazol was established.

Key Words: *afobazol; oxidative stress; glutamate toxicity; neuroprotection*

Afobazol (2-[2-(morpholino)-ethylthio]-5-etoxybenzimidazole dihydrochloride) was synthesized in our institute [4]. Experimental and clinical studies revealed selective anxiolytic properties of this preparation [4]. Afobazol possesses antioxidant property, inhibits activity of neuronal NO-synthase, prevent stress-induced disturbances in the regulation of binding in the benzodiazepine site of the $GABA_A$-receptor complex [1,4, 5]. Injection of this agent in anxiolytic doses to rats before and after ligation of the middle cerebral artery decreased the damaged area [3]. Therefore, three mechanisms (antiradical action, regulation of NO synthesis and GABA-transmission) can underlie the neuroprotective properties of this drug.

Our aim was to evaluate the effects of afobazol on the models of oxidative stress and glutamate toxicity used in studies of neurodegenerative processes [7,9].

MATERIALS AND METHODS

Experiments were carried out on HT-22 cell (immortalized cells from mouse hippocampus) [10]. The cells were incubated in DMEM medium containing 5% FCS. Oxidative stress was modeled by adding 1.5 mM H_2O_2 with 30-min incubation at 37°C in a CO_2-incubator (5% CO_2). Then this medium was replaced with normal (H_2O_2-free) culture medium. After 4 h the cells were washed and their viability was assessed using MTT (3-(4,5-dimethylthiazol-2-il)-2,5-diphenyltetrazolium bromide) test [8]. Glutamate toxicity was modeled by adding 5 mM glutamine acid to the culture medium [10]. After 8-h incubation the cells were washed. Vitality (the survival rate) of the cells was assessed in 24 h [6,10]. Optical density was measured at λ=600 nM with Elisa Reader spectrophotometer. Neuroprotective affect of afobazol at final concentrations of 10^{-8} and 10^{-5} M was tested by adding the drug to the culture medium 24 h and 30 min before and immediately after destructive stimulation. The data were analyzed statistically using Microsoft Excel software and Student's *t* test.

RESULTS

Addition of H_2O_2 to the cell culture significantly decreased neuronal survival rate to 26% (Fig. 1). These changes agree with previous reports [8] and confirm reproducibility of the method. Preventive introduction of afobazol in the examined concentrations enhanced the survival rate of neurons subjected to oxidative stress (Fig. 1, *a*). When the drug was introduced into the culture medium 30 min before H_2O_2, the lower concentration (10^{-8} M) was efficient, and its protective effect was more pronounced (Fig. 1, *a*). Afobazol added into the culture medium immediately after washout from H_2O_2 also exerted the protective effect in all concentrations (Fig. 1, *b*). These data agree with previous data on the protective effect of afobazol during stimulation of ROS generation [4].

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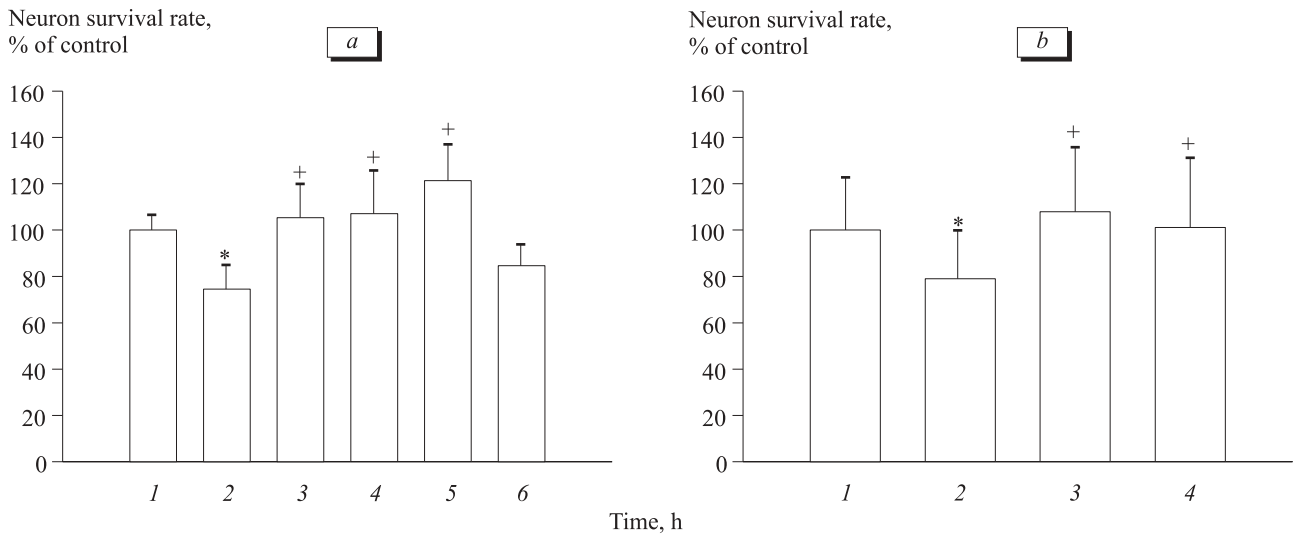

Fig. 1. Protective effect of afobazol (*a*) applied 24 h or 30 min before and (*b*) immediately after oxidative stress in cultured neurons of HT-22 cell line according to MTT test. *a*: *1*) control; *2*) H_2O_2; *3*) afobazol (10^{-8} M) 24 h before stress; *4*) afobazol (10^{-5} M) 24 h before stress; *5*) afobazol (10^{-8} M) 30 min before stress; *6*) afobazol (10^{-5} M) 30 min before stress. *b*: *1*) control; *2*) H_2O_2; *3*) afobazol (10^{-8} M) immediately after stress; *4*) afobazol (10^{-5} M) immediately after stress. $p<0.05$ in comparison with *control and $^{+}H_2O_2$.

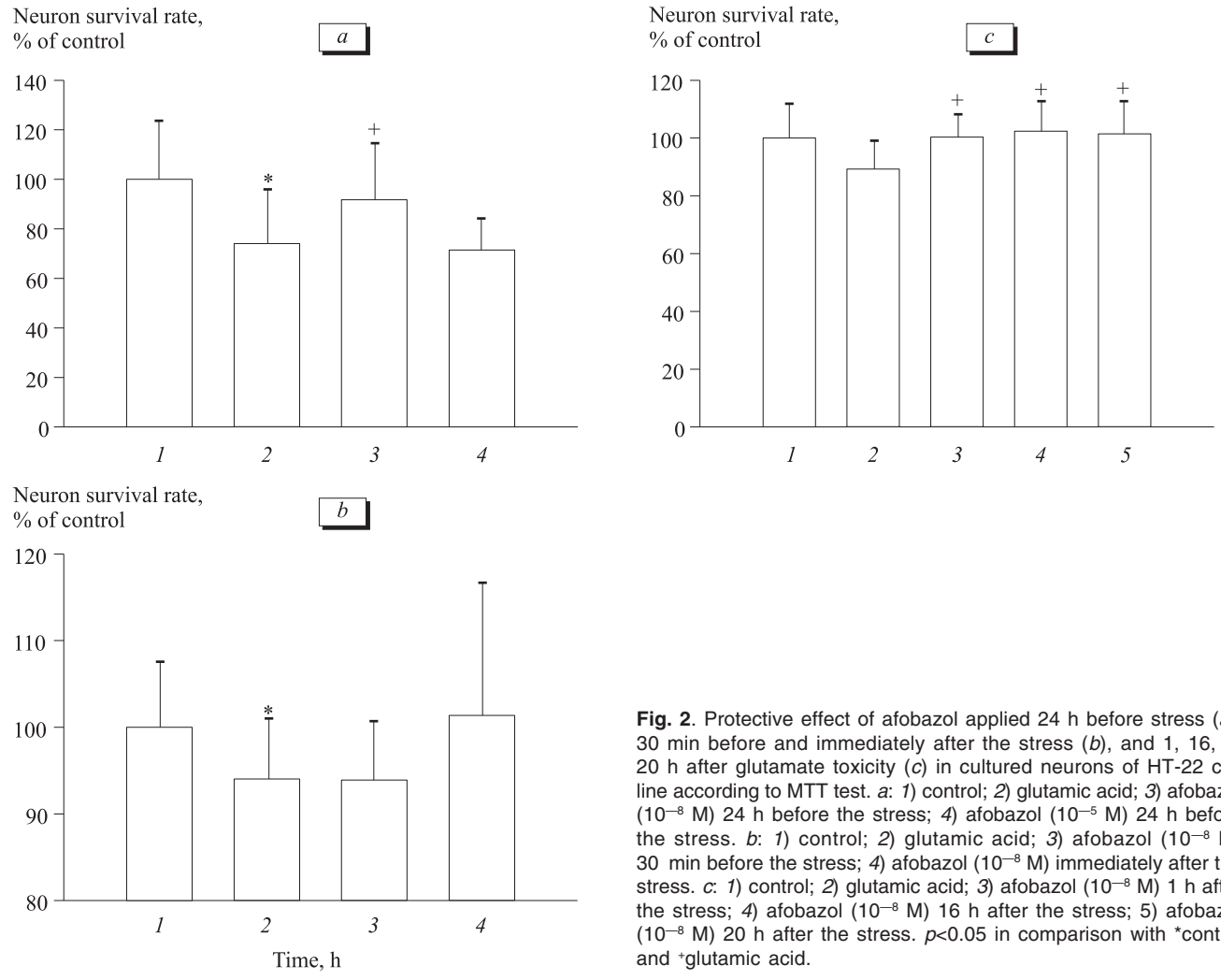

Fig. 2. Protective effect of afobazol applied 24 h before stress (*a*), 30 min before and immediately after the stress (*b*), and 1, 16, or 20 h after glutamate toxicity (*c*) in cultured neurons of HT-22 cell line according to MTT test. *a*: *1*) control; *2*) glutamic acid; *3*) afobazol (10^{-8} M) 24 h before the stress; *4*) afobazol (10^{-5} M) 24 h before the stress. *b*: *1*) control; *2*) glutamic acid; *3*) afobazol (10^{-8} M) 30 min before the stress; *4*) afobazol (10^{-8} M) immediately after the stress. *c*: *1*) control; *2*) glutamic acid; *3*) afobazol (10^{-8} M) 1 h after the stress; *4*) afobazol (10^{-8} M) 16 h after the stress; 5) afobazol (10^{-8} M) 20 h after the stress. $p<0.05$ in comparison with *control and $^{+}$glutamic acid.

Glutamic acid reduced neuron viability by 25%, which agree with previous data [10]. When afobazol (10^{-8} M) was introduced into the medium 24 h before glutamic acid, it exerted a protective effect (Fig. 2, *a*). In the following tests, we used the same concentration of the drug. When afobazol was added to the culture medium 30 min before or immediately after glutamic acid, it was inefficient (Fig. 2, *b*). However, afobazol added 1, 16, or 20 h after damage to cells produced a pronounced protective effect: in these cases, the survival rate was equal to that in the control (Fig. 2, *c*). The protective effect of afobazol after treatment with glutamic acid *in vitro* agrees with previously established *in vivo* protective effect of this drug, when it was applied 3 or 6 h after occlusion of the middle cerebral artery [3].

Thus, when used in concentrations similar to those established in blood plasma after systemic administration of the drug in therapeutic doses, afobazol exerted a direct protective effect during damage to hippocampal neurons produced by H_2O_2 or glutamic acid. The differences in the protective action of anxiolytic afobazol in the used experimental models attest to involvement of different protective mechanisms. Since both oxidative stress and glutamate toxicity are considered as important mechanisms of pathogenesis leading to cerebral ischemia [2], it seems necessary to evaluate the effects of afobazol in the ischemic models *in vivo*.

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