PHARMACOLOGY AND TOXICOLOGY

Study of Anti-Ischemic Effect of Afobazole in Experimental Myocardial Infarction

S. A. Kryzhanovskyi, A. V. Sorokina, V. N. Stolyaruck, M. B. Vititnova, I. A. Miroshkina, I. B. Tsorin, A. D. Durnev, and S. B. Seredenin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 9, pp. 284-287, September, 2010
Original article submitted December 24, 2009

Seven-day treatment of rats with experimental myocardial infarction with afobazole (5-ethoxy-2-[2-morpholino)-ethylthio] benzimidasole dihydrochloride) resulted in shrinkage of the ischemic damage area in the heart, stimulation of reparative processes in the myocardium, and prevention of postinfarction remodeling of the left ventricle. Anti-ischemic effect of afobazole in experimental myocardial infarction is presumably due to its interactions with σ_1 receptors.

Key Words: *myocardial infarction; afobazole; anti-ischemic effect; postinfarction remodeling of the heart*

Afobazole (anxiolytic) has been created and studied at V. V. Zakusov Institute of Pharmacology. The mechanism of action of this drug is intricate because of its capacity to prevent stress-induced reduction of benzodiazepine reception [3] and its interactions with σ_1 melatonin receptors and monoaminoxidase A [3]. The cytoprotective effects of the drug can be due to affinity for σ_1 receptors [2]. The σ_1 receptors are sufficiently widely represented in the CNS and in cells of other organs and tissues, including the myocardium [9]. We previously showed that afobazole, in addition to its anxiolytic activity, is characterized by antiarrhythmic and antifibrillatory effects. The latter effect is presumably explained by its interactions with cardiomyocyte σ_1 receptors [1]. It is known that σ_1 receptor agonists are characterized by anti-ischemic activity [6].

We evaluated the anti-ischemic effect of afobazole in experimental myocardial infarction (MI).

V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** SAK-538@yandex.ru. S. A. Kryzhanovskyi

MATERIALS AND METHODS

Experiments were carried out in outbred male albino rats (180-200 g). The animals were kept in vivarium in accordance with the Order No. 267 of June, 9, 2003, of the Ministry of Health of the Russian Federation "On Laboratory Practice Regulation". Myocardial infarction was induced after Selye. Afobazole was injected intraperitoneally in single daily doses of 5 and 10 mg/kg for 7 days (groups 2 and 3). The first dose was injected directly after coronary artery occlusion. Controls (group 1) were injected with isotonic NaCl in an equivalent volume according to the same protocol. Group 1 consisted of 8 animals, group 2 of 6, and group 3 of 7 rats.

On day 8, the animals were narcotized with urethane (1300 mg/kg intraperitoneally), the hearts were removed and fixed in 10% formalin solution. Transverse sections of the heart (10-15 μ) were sliced with a freezing microtome at two levels (apex and middle of the heart). The sections were stained by the standard methods with gallocyanin and eosin and with picro-

0007-4888/11/1503-0316

fuchsin after van Gieson. Micropreparations were examined in transmitting light. Qualitative visual evaluation of the heart micropreparations was carried out and damaged tissue was described. Quantitative evaluation was carried out using Avtandilov's grid and a ×24 magnifying glass with a ruler of 0.05 mm scale division. The infarction area, thickness of the ventricular wall and septum, and diameter of the left ventricular cavity in the middle section of the heart were measured. Transverse diameters (in arb. units) of cardiomyocyte nuclei were measured using an ocular net in these sections in the apparently intact myocardial zone in order to evaluate the intensity of proliferative processes.

The distribution normality was verified by Shapiro–Wilk test, the significance of changes in the size of infarction zone, thickness of the ventricular wall, and left ventricular diameter and subsequent comparisons were evaluated using one-way Dunnet test. The significance of changes in the nuclear diameters was evaluated using Mann–Whitney test with consideration for multiple comparisons. In order to evaluate the intensity of reparative processes, the nuclei larger than 2 arb. units in diameter (their size served the mode for all samples) were counted. These data were processed by the χ^2 test with consideration for multiple comparisons.

RESULTS

Morphological studies showed that the histological picture of the myocardium in control rats corresponded to that in subacute period of MI. A focus of about 4.1% of the transverse section of the heart was detected in the left ventricular wall (Fig. 1). The morphology of the myocardium was heterogeneous in this group and was characterized by clear-cut zones (infarction, peri-infarction, and intact zones).

An irregularly shaped site with dead cardiomyocytes was detected in the infarction zone. It contained dead cells with lyzed nuclei and hyperoxyphilic sarcoplasm without cross-striation. This focus occupied from $^1/_3$ to $^2/_3$ of the left ventricular wall thickness. Foci of accumulation of polymorphonuclear leukocytes (mature and young forms and their fragments) were seen in the immediate vicinity of the focus. An appreciable portion of the infiltrate cells were lymphomacrophage elements. The infiltration outlined the focus and formed a granulation roll involving the peri-infarction zone. Hemorrhages often intercalated in the infiltration zone. Importantly that connective tissue formation started in the infarction zone and in sites more distant from the focus. This was best of all seen in the micropreparations stained with picrofuchsin after van Gieson. Cardiomyocytes with modified tinctorial characteristics and intact typically stained ones were seen in sites distant from the focus of lesions. Cells with weak cross-striation and large swollen nuclei were rather incident. In addition, vascular reaction manifesting in plethora, stasis, extensive hemorrhages, and perivascular edema was seen in the myocardial tissue.

Proliferative processes in the apparently intact myocardial zone were not intensive; this was seen from low count of cardiomyocyte nuclei >2 arb. units in diameter. In the control, the number of cardiomyocyte nuclei >2 arb. units in diameter was 18%, while the relative content of nuclei ≤2 arb. units in diameter was 82%. After afobazole treatment (10 mg/kg intraperitoneally, 7 days) these values were 65 and 35%, respectively. The thickness of myocardial wall and diameter of the left ventricular cavity are presented in Table 1.

Morphological analysis of the hearts of group 2 rats showed a picture similar to that in the control: an irregularly shaped zone with dead cardiomyocytes with lyzed nuclei and hyperoxyphilic sarcoplasma without cross-striation was seen in the left ventricle. The infarction focus occupied $^1/_3$ to $^2/_3$ of the wall thickness, its size constituting 2.6% of the cross-section area of the heart (Fig. 1).

The intensity of proliferative processes in the myocardium in this group of rats was not high either. The number of cardiomyocyte nuclei with a diameter >2 arb. units did not differ much (p=0.1388) from that in the control.

On the other hand, it is noteworthy that the thickness of the left ventricular wall in group 2 animals was 1.5 times (p=0.0443) greater than in controls (Table 1).

The histological picture of the myocardium in group 3 animals corresponded to subacute period of

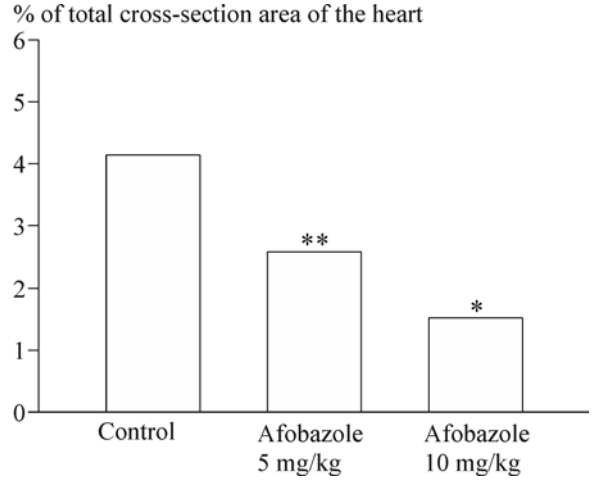

Fig. 1. Effects of afobazole (5 and 10 mg/kg intraperitoneally, 7 days) on the size of experimental MI (% of total area of transverse section of the heart). *p=0.0266, **p=0.1165 compared to the control.

TABLE 1. Effects of Afobazole (5 and 10 mg/kg intraperitoneally, 7 days) on Morphometric Parameters of the Heart in Rats with Experimental MI (*M*±*m*)

Treatment	Number of hearts	Thickness of left ventricular wall, mm	Thickness of ventricular septum, mm	Thickness of right ventricular wall, mm	Diameter of left ventricular cavity, mm
Control	8	0.90±0.19	2.00±0.21	0.88±0.15	5.62±0.41
Afobazole, 5 mg/kg		1.44±0.17	2.30±0.19	1.16±0.14	5.16±0.38
		p=0.0443	*p*=0.2393	*p*=0.1638	*p*=0.3277
Afobazole, 10 mg/kg	7	1.67±0.19	2.30±0.21	1.18±0.15	4.40±0.41
		p=0.0099	*p*=0.2518	*p*=0.1463	*p*=0.0477

Note. *p*: significant difference compared to the control.

MI (cicatrization stage), but, in contrast to the control, was characterized by higher intensity of proliferative processes. This was seen, among other things, from a greater diameter of cardiomyocyte nuclei in the apparently intact zone of the myocardium. The content of cardiomyocyte nuclei with a diameter >2 arb. units in the myocardium of control rats was 17.7% *vs.* 65.5% in group 3 ($p<0.001$). Presumably, so high intensity of proliferative processes in the apparently intact zone of the myocardium led to significant ($p=0.0266$) shrinkage of infarction zone in these animals in comparison with the control (Fig. 1).

A no less important fact is that long-term afobazole therapy statistically significantly prevented the postinfarction remodeling of the left ventricle (Table 1). Left ventricular cavity of group 3 rats was 21.5% less than in the controls ($p=0.0477$).

These data suggest a pronounced anti-ischemic effect of afobazole in acute MI. The drug not only stimulated reparative processes in the myocardium, but also prevented postischemic remodeling of the cardiac muscle. This latter fact seems to be particularly important, because it gives us grounds to expect a lesser risk of heart failure during the postinfarction period in animals treated with afobazole. These results explain to a certain measure the previous experimental data indicating that afobazole prevents the development of heart failure during the postinfarction period [5].

The mechanism of anti-ischemic effect of afobazole is not yet clear. Presumably, it is related to some extent to the agonistic effect of the drug on σ_1 receptors [2]. It is known that σ_1 receptor agonists reduce the size of ischemic damage [6]. It is most likely that σ_1 receptor agonists produce a complex effect including protection of ischemic cells from Ca^{2+} overloading [11], reduction of tonic effect of the sympathetic nervous system on the myocardium [15], inhibition of the cascade of intracellular reactions initiating apoptosis [12] (including the expression of *bcl-2* gene expression [14]), and protection of myocardial cells from free radical aggression [8].

Presumably, suppression of free radical aggression is explained by the capacity of σ_1 receptor agonists to reduce activity of inducible nitroxide synthetase (iNOS) [13]. This enzyme is now regarded as one of the main mediators of ischemic damage to cardiomyocytes. Hyperproduction of iNOS is destructive for cardiomyocytes and initiates dysfunction of the left ventricle, causing its postischemic remodeling and increasing the risk of sudden coronary death [10]. Presumably, inhibition of iNOS activity by σ_1 receptor agonists stimulates the synthesis of constitutive nitroxide synthase (cNOS) by the ischemic myocardium. In contrast to iNOS, cNOS promotes normalization of myocardial contractility and protects the cells from free radical damage [7].

Hence, our data indicate that long-term (7 days) afobazole (10 mg/kg intraperitoneally) treatment promotes shrinkage of ischemic damage area, stimulates proliferative processes in apparently intact myocardium, and prevents postinfarction remodeling of the left ventricle, that is, exhibits anti-ischemic activity, in animals with acute experimental MI.

REFERENCES

1. S. A. Kryzhanovskyi, V. N. Stolyaruck, M. B. Vititnova, *et al.*, *Byull. Eksp. Biol. Med.*, **149**, No. 3, 290-293 (2010).
2. S. B. Seredenine and M. V. Voronin, *Eksp. Klin. Farmakol.*, **72**, No. 1, 3-11 (2009).
3. S. B. Seredenin, T. A. Voronina, G. G. Neznamov, *et al.*, *Vestn. Rossiisk. Akad. Med. Nauk*, No. 11, 3-9 (1998).
4. S. B. Seredenin, G. M. Molodavkin, M. V. Voronin, and T. A. Voronina, *Eksp. Klin. Farmakol.*, **72**, No. 1, 19-21 (2009).
5. I. B. Tsorin, I. P. Palka, and G. G. Chichkanov, *Ibid.*, 41-45.
6. C. T. Ajmo Jr., D. O. Vernon, L. Collier, *et al.*, *Curr. Neurovasc. Res.*, **3**, No. 2, 89-98 (2006).
7. J. W. Calvert and D. J. Lefer, *Cardiovasc. Res.*, **83**, No. 2, 195-203 (2009).

8. B. Halliwell, *J. Neurochem.*, **97**, No. 6, 1634-1658 (2006).
9. M. Novakova, V. Bruderova, Z. Sulova, *et al.*, *Gen. Physiol. Biophys.*, **26**, No. 2, 110-117 (2007).
10. J. R. Somers, P. L. Beck, J. P. Lees-Miller, *et al.*, *Am. J. Physiol. Heart Circ. Physiol.*, **295**, No. 3, H1122-H1131 (2008).
11. K. T. Tchedre, R. Q. Huang, A. Dibas, *et al.*, *Invest. Ophthalmol. Vis. Sci.*, **49**, No. 11, 4993-5002 (2008).
12. K. T. Tchedre and T. Yorio, *Ibid.*, No. 6, 2577-2588.
13. K. Vagnerova, P. D. Hurn, A. Bhardwaj, and J. R. Kirsch, *Anesth. Analg.*, **103**, No. 2, 430-434 (2006).
14. S. Yang, A. Bhardwaj, J. Cheng, *et al.*, *Ibid.*, **104**, No. 5, 1179-1184 (2007).
15. H. Zhang and J. Cuevas, *J. Neurophysiol.*, **87**, No. 6, 2867-2879 (2002).