Role of ADP-Ribosyl Cyclase in the Pathogenesis of Neurological Disorders after Coronary Artery Bypass Surgery and Experimental Ischemia

V. V. Moroz, A. B. Salmina*, A. A. Fursov*,, S. V. Mikhutkina*, K. Y. Linev**, N. S. Mantorova*, and S. V. Shakhmaeva*****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 5, pp. 499-501, May, 2009
Original article submitted September 22, 2008

The pathogenesis of neuronal dysfunction was evaluated from the viewpoint of cellular disturbances in NAD^+ metabolism and changes in activity of NAD^+-utilizing enzymes (*e.g.*, ADP-ribosyl cyclase/CD38). S-100B concentration and CD38 expression on peripheral blood lymphocytes were altered in patients after surgery for coronary heart disease with extracorporeal circulation. These changes in patients during the early postoperative period correlated with variations in CD38 expression on neuronal cells from postischemic rats with cognitive dysfunction.

Key Words: *neuronal dysfunction; ADP-ribosyl cyclase/CD38; NAD^+*

Modern cardiosurgery is impossible without extracorporeal circulation, which maintains patient's life during correction of heart diseases. CNS injury in surgical patients with extracorporeal circulation (ECC) is often observed during the early postoperative period. It is related to circulatory hypoxia, ischemia—reperfusion, progressive energy deficit in neuronal and glial cells, impairment of electrical excitability, and neurotoxic effect of exogenous and endogenous molecules [1].

Studying the mechanisms for damage to neurons and glial cells during CNS injury is an urgent problem of clinical pathophysiology, biochemistry, and molecular medicine. Much attention was paid to evaluation of cellular and molecular mechanisms of neuronal death and dysfunction. However, the development of high-efficacy methods for correction of CNS dysfunction is one of the major problems of modern medicine. Experimental and clinical observations showed that dysfunction of intracellular signal systems in neurons plays the key role in the pathogenesis of these disorders [3,4-6]. These changes are followed by irreversible damage or dysfunction of CNS cells. Clinical signs include sensory, motor, and cognitive dysfunction. They are related to the impairment of synaptic plasticity, induction of programmed death of neurons and glial cells, and dysregulation of synthesis, secretion, and reception of neurotransmitters [1,4,5].

NAD^+ metabolism is one of the factors, which determines the type of changes in neuroglial interactions and sensitivity of cells to apoptosis-inducing agents [3,4].

NAD^+ serves as a coenzyme and substrate for several NAD^+-converting enzymes. However, there are no methods for the diagnostics of CNS cells based on evaluation of NAD^+ metabolism in neuronal and glial cells. It is clear that these disorders originate from neuroglial dysregulation and dam-

Institute of General Resuscitation, Russian Academy of Medical Sciences, Moscow; *Krasnoyarsk State Medical Academy, Federal Agency for Health Protection and Social Development; **Krasnoyarsk Clinical Hospital; ***A. A. Vishnevsky Central Military Clinical Hospital, Russian Ministry of Defense, Arkhangel'skoe, Moscow region, Russia. ***Address for correspondence:*** fursov_alex@mail.ru. A. A. Fursov

0007-4888/09/1475-0570 © 2009 Springer Science + Business Media, Inc.

age to electrically excitable cells (*e.g.*, irreversible injury). Therefore, studying the homeostasis of NAD^+ in CNS cells can serve as a reliable and informative method for the diagnostics of neuronal dysfunction (component of polyorgan insufficiency).

Here we studied the mechanism for changes in viability of neuronal cells due to dysfunction of ADP-ribosyl cyclase.

MATERIALS AND METHODS

The patients were examined after coronary artery bypass surgery. Experimental studies were performed on male outbred albino rats. The concentration of S-100B protein was measured using an enzyme immunoassay kit (BioChemMack). The degree of neurological deficit was evaluated by a neurologist before and 7 days after surgery. Preoperative neurological status, S-100B concentration, and CD38 level served as the control for the group of coronary artery bypass surgery. Acute cerebral ischemia in rats was induced *in vivo* by ligation of the common carotid artery (CCA). The control group consisted of nonischemic animals. The severity of neurological symptoms was evaluated by the international scale for laboratory animals (NSS). The degree of cognitive dysfunction in rats was estimated by the standard test (Morris water maze). CD38 on lymphocytes was detected as follows. Isolated lymphocytes were suspended in 50 μl phosphate buffered saline ($0.5\text{-}1.0\times10^6$ cells per ml). Primary antibodies (CD38 to human lymphocytes, titer 1:50, 3 μl; Sorbent Ltd.) were added to the suspension of lymphocytes. Incubation was performed at 4°C for 1 h. After incubation, the lymphocytes were washed 2 times with 150 μl phosphate buffered saline. The supernatant was removed. Secondary antibodies (FITC-labeled fragments of sheep antibodies to IgG, initial dilution 1:100, 50 μl; Sorbent Ltd.) in phosphate buffered saline and 1% sheep serum were added to the precipitate in a dark room. The cells were incubated at 4°C for 30 min in darkness. After incubation, the cells were washed 2 times with phosphate buffered saline. The suspension of lymphocytes (20 μl) was put on a slide. Luminescence microscopy was performed under a LYuMAM microscope. The calculation was performed relative to 200 lymphocytes with identified $CD38^+$ and CD38 cells. CD38 in brain cells (frozen brain sections) was detected by the standard immunohistochemical study with anti-CD38 antibodies (Sorbent Ltd.). Because of high homology of CD38 in humans, rats, and mice related to high conservatism of the protein sequence, we used antibodies to human antigen in our experiments. Brain sections were incubated with primary antibodies (1:50, 2 h at 37°C) and FITC-labeled anti-mouse secondary antibodies (1:200, 1 h at 4°C). Antigen-antibody complex was visualized by means of luminescence microscopy (×900). We calculated the relative number of cells expressing this antigen in the membrane, perimembrane region, or cytoplasm (diffuse expression).

The results were analyzed by Student's *t* test and T test (SPSS Sigma Stat 3.5, SPSS 13.0, and Excel software).

RESULTS

Evaluation of neurological deficit in patients after coronary artery bypass surgery showed that the severity of dyscirculatory encephalopathy increased from 37.3 to 42.7% ($p<0.05$). Analysis of the neurological status of experimental animals by the NSS scale revealed Horner's syndrome (ptosis, miosis, and enophthalmos) and copticopyramidal syndrome (amblyopia on the ipsilateral to the side of CCA occlusion in combination with contralateral central hemiparesis and hemihypesthesia) 24 h after unilateral extravascular occlusion of CCA ($p<0.001$). Postischemic neurological deficit in the experimental animals was of moderate severity by the NSS scale. It was accompanied by motor dysfunction (impaired locomotor activity, 60% animals) and movement discoordination (dynamic and static ataxia, 90% animals). The degree of neurological deficit progressively increased over the first 48 h after treatment ($p<0.01$), but remained unchanged in the follow-up period. The number of $CD38^+$ cells was similar in the frontal and occipital regions of the brain (17.4±7.4 and 16.8±6.5 cells, respectively). The immunopositive material was diffusely located in the cell cytoplasm. It was also revealed in the perikaryon and along the processes

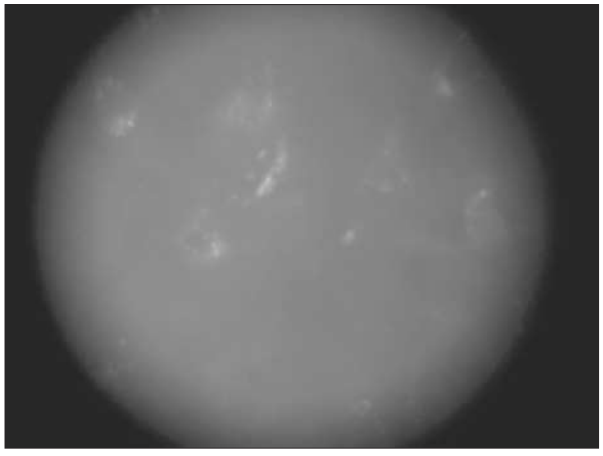

Fig. 1. CD38 expression in rat brain cells, ×1200.

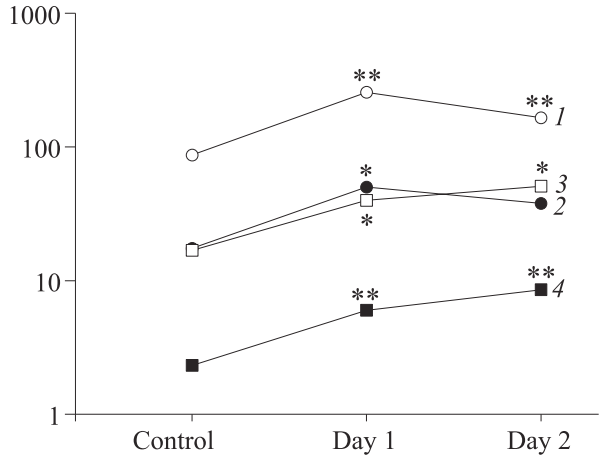

Fig. 2. CD38+ expression on neurons of the frontal (*2*) and occipital regions (*3*) under experimental conditions. CD38+ expression on peripheral blood lymphocytes (*4*) and S-100B protein concentration (*1*) in patients after coronary artery bypass surgery. Ordinate: number of CD38+ per 100 lymphocytes (for CD38); ng/liter (for S-100B). **p*<0.05 and ***p*<0.001 compared to the control.

of nerve cells (Fig. 1). Our results are consistent with published data on the expression of CD38 in brain cells [1,2,7]. CD38 expression was shown to change in the dynamics of ischemic brain injury. The number of CD38+ cells in the frontal and occipital region significantly increased by the 24th hour of the postischemic period. The intracellular distribution of an immunopositive material remained practically unchanged under these conditions. The number of CD38-expressing cells (diffuse expression in the cytoplasm; and expression in the perinuclear region or along the processes of cells) was shown to increase 48 h after experimental occlusion of CCA. The increase in enzyme expression was similar in the frontal and occipital regions. S-100B concentration and CD38 expression on peripheral blood lymphocytes were altered in CHD patients after surgery with ECC. These changes were observed in patients during the early postoperative period and correlated with variations in CD38 expression on neuronal cells from postischemic rats with cognitive dysfunction (Fig. 2).

We conclude that changes in activity of NAD-converting enzyme ADP-ribosyl cyclase/CD38 contribute to variations in electrical excitability and viability of CNS cells. Our results should be taken into account in the development of a neuroprotective method, which is based on the directed modulation of activity or expression of ADP-ribosyl cyclase/CD38 in brain cells.

REFERENCES

1. R. S. Cargill and L. E. Thibault, *J. Neurotrauma*, **13**, No. 7, 395-407 (1996).
2. C. Ceni, N. Pochon, V. Brun, *et al*., *Biochem. J*., **370**, Pt. 1, 175-183 (2003).
3. U. Dirnagl, C. Iadecola, and M. A. Moskowitz, *Trends Neurosci*., **22**, No. 9, 391-397 (1999).
4. G. B. Fox, L. Fan, R. A. Levasseur, and A. I. Faden, *J. Neurotrauma*, **15**, No. 8, 599-614 (1998).
5. K. Hattori, H. Lee, P. D. Hurn, *et al*., *Stroke*, **31**, No. 8, 1939-1944 (2000).
6. M. P. Mattson, J. N. Keller, and J. G. Begley, *Exp. Neurol*., **153**, No. 1, 35-48 (1998).
7. M. Yamada, M. Mizuguchi, N. Otsuka, *et al*., *Brain Res*., **756**, Nos. 1-2, 52-60 (1997).