EPR Investigation of Antioxidant Protection and Production of Nitric Oxide in Rat after Spinal Cord Injury

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1. INTRODUCTION

Nitric oxide (NO) is known to be a paramount signaling molecule modulating the physiological functions of the organism and the cell metabolism. Its role is documented for the central and autonomous nervous systems, for cardiovascular function and blood supply to the brain and the heart, where deviations in NO level may incur risks of stroke and infarction. The NO system is also essential in adaptation to environmental changes and external conditions such as physical load. There is also an opposite point of view, according to which an excess of NO is a compensatory factor that helps to maintain tissue perfusion and provides antiarrhythmic effect during reperfusion.

The high frequency of spinal injury (SCI) combined with the complexity of the pathogenesis, and the lack at present adequate methods of treatment and rehabilitation of patients with consequences of spinal cord (SC) render this problem beyond the purely medical aspects. Great interest attracts the participation in the mechanisms of development of various pathological conditions of the body free radical compounds NO. It is known that major damaging factor during the development of processes of apoptosis is the peroxynitrite (ONOO-), which is formed when NO interacted with superoxide (O2-). Dismutation of superoxide by cytosolic enzyme Cu,Zn-COD (superoxide dismutase) is the primary and primary protection against free-radical oxidation processes. During modeling of SCI it is studied the content of copper, which is an indicator of activity of COD, and also the production of NO in the SC.

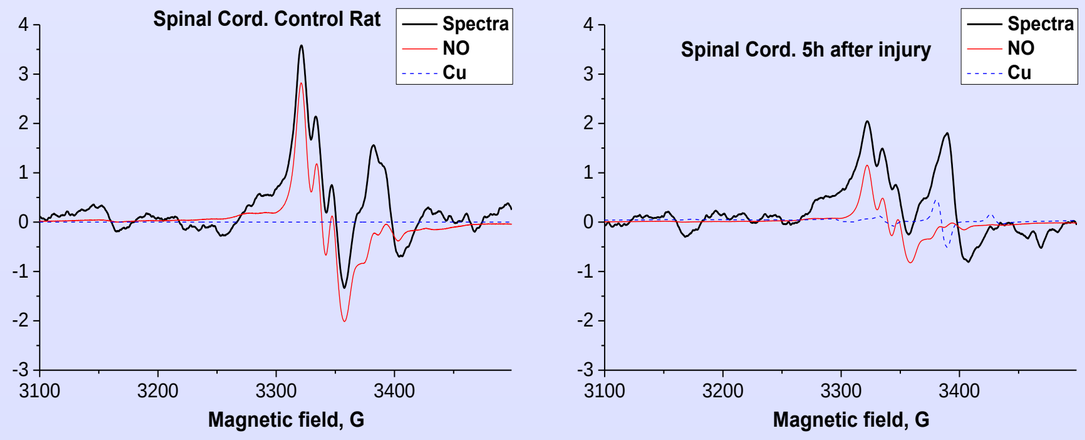

Fig. 1. EPR spectra of spinal cord of healthy rat and rat after SCI. The signals from: tissue sample, complex (DETC)2-Fe2+-NO, complex (DETC)2-Cu2+.
Temperature is 77o K. g=2.038.
The rats were injected with (DETC)2-Fe2+ - citrate.

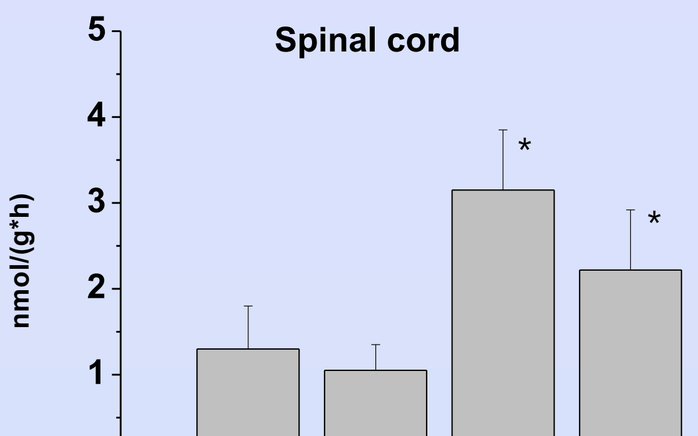

Fig. 2. The Intensity of EPR signal from complex (DETC) 2-Fe2+ - NO from spinal cord tissues in 1 and 3 days and in chronic period after spinal injury.

2. MATERIALS AND METHODS

We used a model of spinal cord injury according to the modified method of A. Allen. Rats of both sexes, weighing 200±20 g, aged up to 1 year (n=41) were used for the study. The standard open vertebral-SCI was applied by the method of TH9 vertebra. The control of the injury was muscle contraction in the lower extremities and visualization of the contusion site SC. We studied the tissue samples of the spinal cord, liver and heart in intact animals and in different periods of traumatic disease of the spinal cord by EPR spectroscopy using the method of spin traps. Tissue samples were extracted in intact animals, as well as in acute (1, 3 days after SCI) and chronic (7 days and 3 months after SCI) periods of traumatic disease. The weight of the samples was 100 mg.

Recently, one of the most effective methods for the detection and quantification of NO in biological tissues is method of the electron paramagnetic resonance (EPR) using the technique of spin traps, it allows to detect NO in low concentrations. We used the complex of Fe2 + with diethyldithiocarbamate ((DETC)2-Fe2+) as a spin trap. The records were carried out on EPR spectrometer X-band firm "Bruker" ER 200E SRC (9.50 GHz). The complex of the spin trap with NO ((DETC)2-Fe2+ -NO) is characterized by easily recognizable EPR spectrum at the value of the g-factor g = 2.038 and triplet hyperfine structure. The amplitude of the EPR spectra was always normalized to the weight of the sample (details of the EPR signal measurement technique described earlier).

The statistical software SigmaStat32 was used. The statistical significance criterion was $p < 0.05$.

3. RESULTS

The figure 1 shows the EPR spectrum of the spinal cord tissue of intact and spinal rats. On this spectrum there is typical triplet signal from the complex (DETC)2-Fe2+-NO at the value of the g-factor, 2,038. . After 5 hours of SCI there is a decrease of NO production in the tested tissues (Fig. 2) and remains low up to 1 day. 3 days after SCI level of NO production in SC increases in 2,5 times. In a chronic period of SCI observed decrease in the level of NO production in the tissues of the heart, but in the SC levels of NO production in chronic period of traumatic disease was significantly more control level (Fig. 2).

We used the method of EPR spectroscopy also as method for estimation of the balance of systems (DETC)2-Cu and (DETC)2-Fe2+-NO (NO/Cu). Figure 3 show the decrease of level of Cu. The ratio of NO/Cu in SCI in intact rats averaged 1:80, which apparently helps to prevent the formation of peroxynitrite. It was found that 3 days after SCI, when the level of NO production in SC was on average 2.5 times more than in intact animals, also increases the concentration of (DETC)2-Cu; and the ratio of NO/Cu in this period has averaged 1:50. In chronic period, the ratio NO/Cu was only 1:3. Thus, the dynamics of the intensity of formation of NO after SCI indicates its possible role as an inducer of apoptosis in the tissue of the damaged SC, and generalized activation of NO-ergic stress-limiting system in the early stages of SCI.

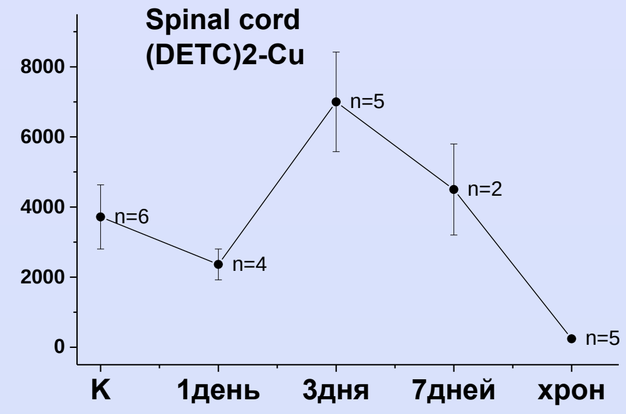

Fig. 3. The relative content of Cu in spinal cord of rats after 5 various times of SCI.

4. CONCLUSION

The dynamics of the intensity of formation of NO after SCI indicates its possible role as an inducer of apoptosis in the tissue of the damaged SC, and generalized activation of NO-ergic stress-limiting system in the early stages of SCI.

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Supported by RFBR (Grant No. 17-04-01746).