METHODS

Oxidative Stress Monitoring in Biological Samples by Bioluminescent Method

N. N. Remmel'*, N. M. Titova, and V. A. Kratasyuk*,****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 8, pp. 238-240, August, 2003
Original article submitted November 22, 2002

The integral bioluminescent biotest with lyophilized fluorescent bacteria was used for monitoring of LPO processes in tissue extracts and serum of rats exposed to stress. A relationship between the content of MDA (LPO indicator) and fluorescence of bacteria was observed in all biological samples.

Key Words: *bioluminescent analysis; oxidative stress; lipid peroxidation; malonic dialdehyde*

Oxidative stress and high intensity of LPO processes are regarded as metabolic disorders, which are to be corrected; LPO activation is associated with many diseases or develops during exposure to adverse factors [8]. Many methods were proposed for studies of the formation and destruction of peroxide compounds [10]. The test used most often is measurement of TBA-active substances [11]. Aldehyde substrate of luciferase is a metabolite regulating fluorescent activity of bacteria *in vivo*. LPO is considered as an alternative to the existing mechanism of aldehyde substrate formation [7]. The fluorescence intensity and activity of luciferase increases during culturing of bacteria or LPO induction by bivalent iron ions. Hence, we can expect that LPO products in biological samples will also stimulate fluorescence of bacteria.

We investigated the possibility of using fluorescent bacteria for integral monitoring of LPO in biological samples in oxidative stress.

MATERIALS AND METHODS

Extracts of the liver, kidney, brain tissues and serum of male Wistar rats served as biological samples. Stress was induced by water immersion (group 1) and by exposure to PC monitor (group 2). Biological samples from rats not exposed to stress factors served as the control.

Lyophilized fluorescent *Photobacterium phosphoreum* were used in the study [4]. Bioluminescence intensity was registered on a BLM-8801 bioluminometer. A studied sample was put into experimental cuvette instead of normal saline. The bacterial index was calculated using the following formula: $BI=I_O/I_K$ [2,3]. The concentration of MDA was measured by the spectrophotometric method by reaction with TBA [9]. The mean values and standard deviation were calculated by Statistica 5.0 software. Analysis of correlations was carried out using Pierson's coefficient.

RESULTS

The intensity of bacteria fluorescence depends on intracellular concentration of luciferase and on the concentrations of its substrates (reduced flavin mononucleotide, aliphatic aldehyde, and oxygen). Intensification of LPO processes in bacteria leads to intensification of fluorescence (Fig. 1), which is in line with published data [7].

Addition of tissue extracts or sera from rats exposed to stress to the bacterial suspension intensified

*Institute of Biophysics, Siberian Division of Russian Academy of Sciences, Krasnoyarsk; **Krasnoyarsk State University. ***Address for correspondence:*** lucifer@ibp.ru. Remmel' N. N.

0007-4888/03/1362-0209$25.00

fluorescence in comparison with the control. Normal saline had no effect on fluorescence intensity. In parallel, MDA concentrations in tissue extracts and sera were measured for evaluating the intensity of LPO processes. Exposure of rats to the PC monitor led to an increase in LPO intensity and MDA concentrations in all tissues, the most pronounced shifts were observed in the liver (Fig. 2). Exposure of rats to water immersion stress led to an increase of MDA concentration in comparison with the control and with group 2. The concentration of MDA in the serum of group 2 animals slightly increased in comparison with the control and the values were lower than in tissue extracts. Measurements of the intensity of bacterial fluorescence produced similar results. Addition of tissue extract or serum of rats to the bacteria resulted in an increase of bacterial fluorescence, this increase was more pronounced after addition of biological samples from experimental rats than from controls. The highest bacterial index was recorded after addition of liver extracts to the bacteria (Fig. 3). BI values for the serum and kidney and brain extracts were virtually the same and significantly higher than in the control. Analysis of correlations showed a significant relationship between BI values and MDA concentrations (Fig. 3), the coefficient of correlation being 0.86 ($p<0.001$).

The time needed for attaining the maximum fluorescence also depended on MDA concentrations in the studied samples: the higher the aldehyde content, the more rapidly peaked fluorescence. It is known that the rate of penetration through the bacterial membrane for aliphatic aldehydes depends on aldehyde concentration and length of its chain [5]. Two mechanisms of stimulating the bacterial fluorescence by biological samples are proposed: MDA penetration through the membrane and participation in the luciferase reaction as a substrate and accumulation of LPO products in the bacteria under the effect of active radicals present in the samples.

Hence, the findings indicate that increased MDA concentration in biological samples after oxidative stress determines enhanced bacterial fluorescence after addition of the samples to bacterial suspension.

Clinical practice and experimental studies need simple and cheap methods for detection of oxidative stress. Bioluminescent methods with the use of fluorescent bacteria or bacterial enzyme systems are rapid, simple, highly sensitive, and require the minimum volumes of the studied sample. Our data indicate that bioluminescent bacteria can be used for monitoring of the level of LPO products (MDA) in biological samples in oxidative stress. BI values clearly demonstrate the level of MDA, though the most close values of the parameters do not always fall within the confidence interval because of high sensitivity of the method. The

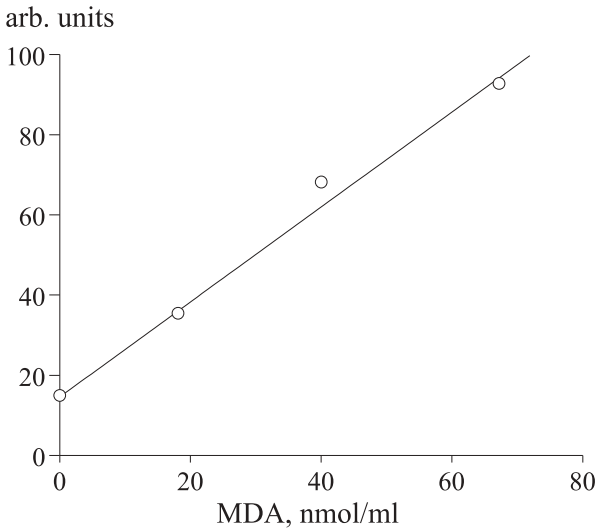

Fig. 1. Relationship between the intensity of bacterial fluorescence and MDA concentration *in vivo*.

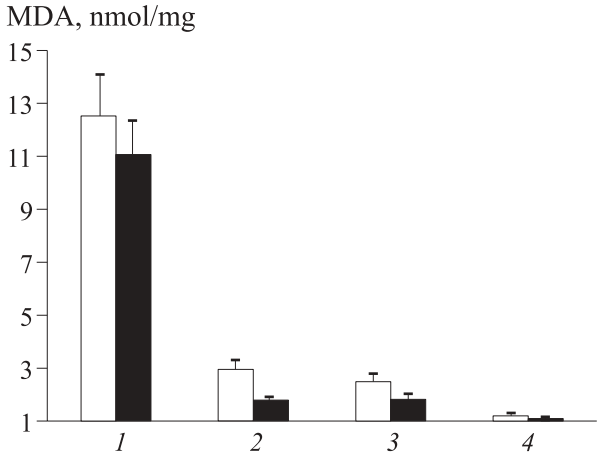

Fig. 2. Relative values of MDA in tissue extracts and serum of rats exposed to water immersion stress (light bars) and to PC monitor radiation (dark bars). Here and in Fig. 3: *1*) liver; *2*) kidneys; *3*) brain; *4*) serum.

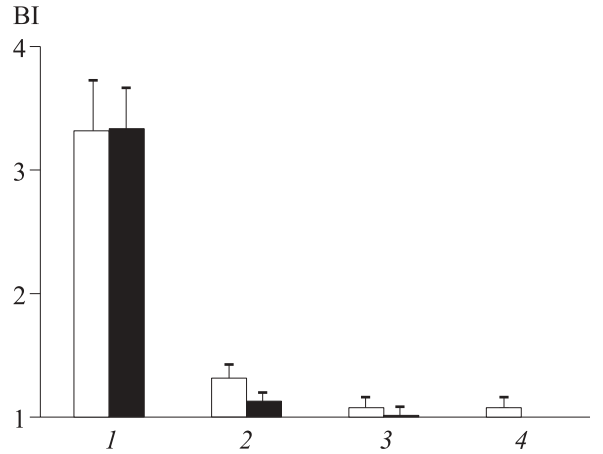

Fig. 3. Relative values of bacterial index (BI) in rats exposed to water immersion stress (light bars) and to PC monitor radiation (dark bars).

proposed integral method can be used in experiments not requiring precise measurements of the analyzed parameters.

The study was supported by grants "Universities of Russia" (UR.11.01.016) from the Ministry of Education of the Russian Federation, from Krasnoyarsk Territorial Research Foundation No. 10F 233C, and the REC-002 grant of the program "Basic Research and Higher Education" of Ministry of Education of Russian Federation and American Foundation for Civil Research and Development for Independent States of the Former Soviet Union.

REFERENCES

1. L. V. Beriya, A. D. Ismailov, and V. S. Danilov, *Biokhimiya*, **56**, No. 3, 477-485 (1991).
2. I. I. Gitel'zon, V. A. Kratasyuk, V. N. Lopatin, *et al.*, *Ecological Biophysics* [in Russian], Vol. 1, Moscow (2002), pp. 8-21.
3. V. A. Kratasyuk and I. I. Gitel'zon, *Uspekhi Mikrobiol.*, No. 21, 3-30 (1987).
4. A. M. Kuznetsov, E. K. Rodicheva, and E. V. Shilova, *Biotekhnologiya*, No. 9, 57-61 (1996).
5. V. A. Marganiya, Yu. A. Malkov, V. S. Danilov, *Mikrobiologiya*, No. 4, 79-84 (1989).
6. A. Yu. Sobolev, A. D. Ismailov, and V. S. Danilov, *Biokhimiya*, **54**, No. 12, 477-485 (1989).
7. A. Berkovich, U. Cogan, and S. Ulitzur, *J Biolumin. Chemilumin.*, **3**, No. 3, 125-129 (1991).
8. C. E. Cross, E. T. Halliwell, E. T. Borish, *et al.*, *Ann. Intern. Med.*, No. 107, 526-545 (1987).
9. H. Esterbauer and K. H. Cheeseman, *Methods Enzymol.*, **186**, 407-421 (1990).
10. J. V. C. Gutteridge and B. Halliwell, *Trends Biochem. Sci.*, No. 2, 129-135 (1990).
11. B. Halliwell and S. Chirico, *J. Clin. Nutr.*, **57**, 715-725 (1993).