ISSN 0006-2979, Biochemistry (Moscow), 2008, Vol. 73, No. 1, pp. 76-79. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © A. V. Makeeva, T. N. Popova, L. V. Matasova, I. N. Yama, 2008, published in Biokhimiya, 2008, Vol. 73, No. 1, pp. 93-97.

Effects of Lipoic Acid on Citrate Content, Aconitate Hydratase Activity, and Oxidative Status during Myocardial Ischemia in Rats

A. V. Makeeva*, T. N. Popova, L. V. Matasova, and I. N. Yama

Faculty of Biology and Soil Sciences, Voronezh State University, Universitetskaya pl. 1, 394006 Voronezh, Russia; fax: (4732) 208-755; E-mail: voloskova@bio.vsu.ru

Received February 20, 2007
Revision received July 31, 2007

Abstract—The effects of lipoic acid on intensity of free radical reactions, citrate content, and aconitate hydratase during myocardial ischemia have been investigated. Treatment with lipoic acid normalized biochemiluminescence parameters and citrate level, which were increased in the myocardial pathology. Treatment with lipoic acid also increased specific activity of aconitate hydratase, which was decreased in myocardium and blood of animals with myocardial ischemia. Administration of lipoic acid decreased DNA fragmentation observed during myocardial ischemia. The data suggest that lipoic acid can be effectively used as a cardioprotector preventing the development of free radical oxidation during myocardial ischemia.

DOI: 10.1134/S0006297908010112

Key words: myocardial ischemia, lipoic acid, citrate, aconitate hydratase

According to modern concepts, membrane damaging processes in the body represent a key mechanism in the development of various diseases including cardiac pathologies. Their manifestation depends on lipid peroxidation (LPO) and the system of antioxidant defense [1, 2]. Cells deficient in the antioxidant defense are especially susceptible to signals triggering apoptosis [3, 4]. Information on biochemical signal-transducing pathways of apoptosis rationalized effective use of antioxidant therapy [5]. There is evidence indicating that one of the targets or free radical attack is aconitate hydratase (AH; EC 4.2.1.3); this enzyme catalyzes conversion of citrate into isocitrate [6]. Activation of free radical oxidation causes changes in regulation of AH activity and results in the decrease of enzyme activity and accumulation of citrate, exhibiting properties of a low molecular weight antioxidant due to chelation of Fe^{2+} [7].

Lipoic acid is an effective antioxidant used in treatment of hepatic pathologies, neurodegenerative diseases, and diabetes mellitus [8, 9]. In the body, lipoic acid forms a dynamic redox system involved in acyl group transfer within multicomponent enzymatic systems. It is a cofactor involved in oxidative decarboxylation of α-keto acids. The antioxidant effect of lipoic acid is associated with the presence of two thiol groups and its ability to bind radical molecules and free iron ions [10, 11]. Lipoic acid not only has its own antioxidant potential, but it also supports functioning of other antioxidant components in the body [12].

The goal of this study was to investigate the effects of lipoid acid on intensity of free radical reactions, citrate content, and cardiac and blood serum AH activity in rats with experimental myocardial ischemia.

MATERIALS AND METHODS

Male albino laboratory rats (*Rattus rattus* L.) weighing 150-200 g were used in experiments. The rats were subdivided into four experimental groups: I) control; II) experimental group of animals treated with 0.1% adrenaline at dose 0.15 ml per 100 g body mass [13] to model myocardial ischemia; animals of the third (III) and the fourth (IV) groups were treated after experimental ischemia with different doses of lipoic acid, 35 and 70 mg/kg, respectively. Hearts removed from narcotized rats were homogenized in three volumes of cooled isolation medium (0.1 M Tris-HCl buffer, pH 7.8, containing

Abbreviations: AH) aconitate hydratase; AsAT) aspartate aminotransferase; CD) conjugated dienes; CPK-MB) myocardial MB isoform of creatine phosphokinase; LPO) lipid peroxidation; ROS) reactive oxygen species.

* To whom correspondence should be addressed.

1 mM EDTA, and 1% β-mercaptoethanol). Homogenate centrifuged at 7000*g* 10 min was then used for analysis. Blood taken from the heart was thermostatted at 37°C for 0.5 h, centrifuged at 3000*g* for 10 min, and the resulting serum was used for subsequent studies.

Activities of aspartate aminotransferase (AsAT) and the myocardial MB isoform of creatine phosphokinase (CPK-MB) were assayed using standard kits (Bio-La-Test, Russia). The level of primary LPO products, conjugated dienes (CD), was evaluated spectrophotometrically at 233 nm [14]. The intensity of free radical processes in blood serum and cardiac tissue was determined by the method of Fe^{2+}-induced chemiluminescence. DNA was isolated by the phenol–chloroform method. DNA fragmentation was detected by electrophoresis in agarose gel [15] applying 10 μg DNA per lane. The molecular mass markers, MassRuler, were from Fermentas (USA). Citrate content was determined by the method of Natel'son [16]. AH activity was determined spectrophotometrically at 240 nm [17], and protein content was determined by the Lowry method [18].

The data were treated using Student's *t*-criterion; differences were considered as statistically significant at $p < 0.05$.

The following reagents were used in this study: Tris-HCl buffer and EDTA from Reanal (Hungary); citrate from Sigma (USA); lipoic acid from ICN (Germany); other reagents of chemical pure or pure for analysis grades from Russian suppliers.

RESULTS AND DISCUSSION

The development of myocardial ischemia was evaluated by blood serum AsAT and CPK-MB activities. There was 6-fold increase of AsAT and CPK-MB activity in blood serum one day after administration of 0.1% adrenaline solution. Treatment of ischemic animals with lipoic acid at the doses 35 and 70 mg/kg decreased activities of AsAT and CPK-MB (Table 1). Since activity of these enzymes in blood serum reflects the degree of cardiomyocyte cytolysis, one may conclude that lipoic acid exerts a positive effect on myocardium during experimental ischemia.

The development of myocardial ischemia in rats was accompanied by 3.7- and 4.2-fold increase of CD in heart and blood serum, respectively (Table 2). These data suggest activation of lipid peroxidation and accumulation of LPO products.

Analysis of spectral characteristics of lipids extracted into a heptane phase from myocardial subcellular fractions showed the increase of optical density at 230-268 nm with a peak at 233 nm suggesting the presence of conjugated dienes and lipid hydroperoxides in the analyzed samples. Administration of lipoic acid to the ischemic rats caused 1.3- and 2.2-fold decrease in heart CD of animals of groups III and IV compared with animals of group II (treated with adrenaline only). Similar changes were also determined in blood serum (Table 2). Light sums of chemiluminescence (*S*) and intensity of maximal flash (I_{max}), reflecting intensity of free radical oxidation significantly increased in heart and blood serum of animals subjected to experimental myocardial ischemia. Ischemia caused the increase of tangent of the slope angle of the kinetic curve ($\tan\alpha_2$) characterizing total antioxidant activity. This suggests that ischemia triggers some compensatory mechanisms responsible for the decrease of free radical oxidation in the cell. Administration of lipoic acid to the ischemic animals caused a dose-dependent decrease in parameters of biochemiluminescence in heart and blood samples (Table 2). These results are consistent with literature data showing that lipoic acid can bind hydroxyl radical, peroxynitrite, and singlet oxygen, and the reduced form of lipoic acid binds superoxide radical [19].

The development of ischemia was accompanied by DNA fragmentation, and the DNA fragments formed the typical "apoptotic ladder" (figure). Some authors believe that such fragments appear as a result of action of apoptosis-specific nucleases activated under conditions of oxidative stress [20]. In the region of low molecular masses there was also a mobile band corresponding to degraded DNA typical for necrosis [21]. Administration of lipoic acid to ischemic rats resulted in the decrease of the degree of DNA fragmentation (figure), suggesting anti-apoptotic effect of lipoic acid.

Under conditions of oxidative stress, AH is now considered as a sensitive and critical target for reactive oxygen species [6, 22]. The development of myocardial ischemia was accompanied by 2.9- and 5.5-fold decrease of specific AH activity in myocardium and serum, respectively (Table 3). This decrease in AH activity may be attributed to possible damage to iron-sulfur clusters of this enzyme

Table 1. Activity of aspartate aminotransferase (AsAT) and creatine phosphokinase MB (CPK-MB) under normal conditions, during myocardial ischemia, and after administration of lipoic acid

Group of animals	Activity, U/liter	
	AsAT	CPK-MB
I	14.7 ± 0.58	115 ± 4.6
II	85.7 ± 3.43*	647 ± 17.2*
III	61.8 ± 2.47*	498 ± 15.4*
IV	58.9 ± 2.36*	359 ± 13.6*

* Differences were statistically significant ($p < 0.05$) compared with control.

Table 2. Effect of lipoic acid on the content of conjugated dienes and parameters of biochemiluminescence during the development of experimental myocardial ischemia in rats

Group of animals	Biological material	Level of conjugated dienes, μmol/liter	S, mW·sec	I_{max}, mW	tanα_2
I	heart	17.73 ± 0.71	14.54 ± 0.58	2.43 ± 0.09	1.80 ± 0.07
	serum	7.50 ± 0.29	27.50 ± 1.09	5.25 ± 0.21	1.08 ± 0.04
II	heart	66.38 ± 2.65*	25.02 ± 1.01*	9.44 ± 0.37*	5.96 ± 0.23*
	serum	31.52 ± 1.26*	53.34 ± 2.13*	8.70 ± 0.34*	2.78 ± 0.11*
III	heart	50.91 ± 2.04*	17.59 ± 0.69*	3.63 ± 0.14*	1.78 ± 0.07
	serum	21.92 ± 0.87*	36.39 ± 1.45*	5.24 ± 0.19	1.53 ± 0.06*
IV	heart	30.48 ± 1.21*	15.78 ± 0.63*	2.38 ± 0.08	1.07 ± 0.04*
	serum	12.37 ± 0.49*	28.13 ± 1.12	3.27 ± 0.13*	0.92 ± 0.03

* Differences were statistically significant ($p < 0.05$) compared with control.

Table 3. Activity of aconitate hydratase in myocardium and blood serum of control rats and rats subjected to myocardial ischemia and treatment with lipoic acid

Group of animals	Aconitate hydratase activity			
	heart		blood serum	
	U/g wet mass	U/mg of protein	U/ml	U/mg of protein
I	2.38 ± 0.09	0.198 ± 0.007	1.12 ± 0.04	0.060 ± 0.0024
II	1.27 ± 0.05*	0.068 ± 0.002*	0.36 ± 0.01*	0.011 ± 0.0004*
III	1.86 ± 0.07*	0.087 ± 0.003*	0.60 ± 0.02*	0.026 ± 0.0011*
IV	2.29 ± 0.09	0.166 ± 0.006*	0.96 ± 0.03*	0.045 ± 0.0018*

* Differences were statistically significant ($p < 0.05$) compared with control.

Table 4. Content of citrate in heart and blood serum of control rats and rats subjected to myocardial ischemia and treatment with lipoic acid

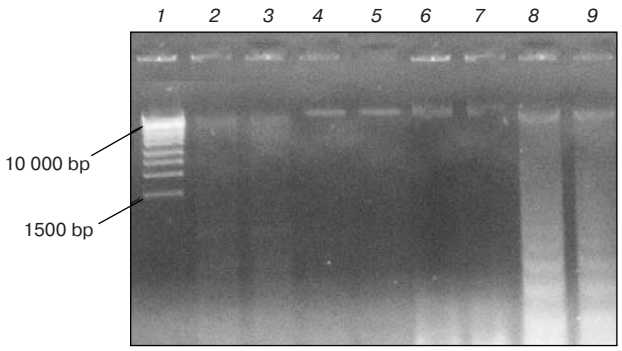
Group of animals	Citrate content, μmol/ml	
	heart	blood serum
I	0.203 ± 0.008	0.569 ± 0.023
II	0.734 ± 0.029*	0.810 ± 0.032*
III	0.589 ± 0.024*	0.623 ± 0.025*
IV	0.379 ± 0.015*	0.543 ± 0.022

* Differences were statistically significant ($p < 0.05$) compared with control.

by increased concentrations of free radicals (typical for this pathology) followed by inactivation of AH molecules [23]. It has been demonstrated earlier that aconitase activity decreases in some disorders, including cardiovascular diseases [24].

Changes in AH activity seen in myocardial ischemia were coupled to accumulation of citrate. In the ischemic animals, citrate content in heart and serum was 3.6- and 1.4-times higher than in control (Table 4). The increase of citrate content in myocardial ischemia may have adaptive importance due to chelation properties of citrate with respect to bivalent iron ions, which are catalysts of free radical processes [25].

Administration of lipoic acids at doses 35 and 70 mg/kg caused 1.3- and 2.4-fold increase of cardiac AH activity in the animals of groups III and IV, respectively, compared with group II; in blood serum AH activity in animals of groups III and IV increased by 2.4 and 4.1



Electrophoregram of rat heart DNA: *1*) molecular mass markers; *2*, *3*) rats of group III; *4*, *5*) rats of group I (control); *6*, *7*) rats of group IV; *8*, *9*) rats of group II

times compared with animals of group II. Cardiac AH activity calculated per g wet mass was also higher in ischemic animals treated with lipoic acid (Table 3). It is possible that normalization of AH activity is associated with the decrease of free radical oxidation. This was also accompanied by decreased citrate content in the heart and blood serum (after administration of lipoic acid to ischemic animals compared with untreated ischemic animals of group II) (Table 4).

Thus, results of this study indicate that administration of lipoic acid may correct oxidative status of myocardium and blood of rats with myocardial ischemia due to the decrease of free radical processes and accompanying reduction of mobilization of the antioxidant system. It has also been shown that treatment with lipoic acid decreased DNA fragmentation stimulated by myocardial ischemia. Treatment with lipoic acid also decreased citrate content and normalization of aconitate hydratase activity.

This work was financially supported by the Ministry of Education and Science within the Program "Development of Scientific Potential of Higher School" (RNP 2.1.1.4429).

REFERENCES

1. Litvitsky, P. F., Sandrikov, V. A., and Demurov, E. A. (1994) *Adaptive and Pathogenic Effect of Reperfusion and Reoxygenation of Myocardium* [in Russian], Meditsina, Moscow.
2. Bilenko, M. V. (1989) *Ischemic and Reperfusion Injuries of Organs* [in Russian], Meditsina, Moscow.
3. Agid, Y. (1995) *Bull. Acad. Natl. Med.*, **179**, 1193-1203.
4. Cafforio, P., Romito, A., and Grizzuii, M. A. (1996) *Recent. Prog. Med.*, **87**, 366-373.
5. Philpott, K. L., Me Carthy, M. J., and Backer, D. (1996) *Eur. J. Neurosci.*, **8**, 1906-1915.
6. Gardner, P. R., Nguyen, D. M., and White, C. W. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 12248-12252.
7. Skulachev, V. P. (1996) *Quant. Rev. Biophys.*, **29**, 169-203.
8. Bustamante, J., Lodge, J. K., Marcocci, L., Tritschler, H. J., Packer, L., and Rihn, B. H. (2001) *Int. Med. J.*, **2**, 133-141.
9. Kishi, Y., Schmelser, J. D., and Yao, J. K. (1999) *Diabetes*, **48**, 2045-2051.
10. Deneke, S. M. (2000) *Curr. Top. Cell Regul.*, **36**, 151-180.
11. Self, W. T., Tsai, L., and Stadtman, T. C. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 12481-12486.
12. Trent, W. N. (1997) *Alternative Med. Rev.*, **2**, 177-183.
13. Nepomnyaschikh, L. M., Lushnikova, E. L., and Semenov, D. E. (2002) *Byul. Eksp. Biol. Med.*, **134**, 219-226.
14. Stal'naya, I. D., and Garishvili, T. G. (1977) *Modern Methods in Biochemistry* [in Russian], Meditsina, Moscow.
15. Kalinina, T. S., Bannova, A. V., and Dygalo, N. N. (2002) *Byul. Eksp. Biol. Med.*, **134**, 641-644.
16. Afanas'ev, V. G., Zaitsev, V. S., and Vol'fson, T. I. (1973) *Lab. Delo*, **1**, 115-116.
17. Guilbault, G. G. (1976) *Handbook of Enzymatic Methods of Analysis*, N-Y.
18. Lowry, O. H., Rosebrough, N. J., and Farr, A. L. (1951) *J. Biol. Chem.*, **194**, 265-271.
19. Packer, L. (1998) *Drug. Metab. Rev.*, **30**, 245-275.
20. Muller, K. (1992) *Eur. J. Pharmacol.*, **226**, 209-214.
21. Yarylin, A. A. (1998) *Patol. Fiziol. Eksp. Ther.*, No. 2, 38-48.
22. Murakami, K., and Yoshino, M. (1997) *Biochem. Mol. Biol. Int.*, **41**, 481-486.
23. Gardner, P. R., and Raineri, J. (1995) *J. Biol. Chem.*, **270**, 11399-13405.
24. Sadek, H. A., Humphries, K. M., Szweda, P. A., and Szweda, L. I. (2002) *Arch. Biochem. Biophys.*, **406**, 222-228.
25. Yakobson, M. G., Antonov, A. R., Golovatyuk, A. V., and Efremov, A. V. (2001) *Byul. Eksp. Biol. Med.*, **132**, 502-505.