PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

Effects of Age and Ischemia on Levels of Lipoperoxides and Lipid-Soluble Antioxidants in the Human Heart

A. V. Lebedev,* S. A. Afanas'ev, E. D. Alekseeva, M. N. Makarova,*
A. M. Chernyavskii, Sh. D. Akhmedov, M. V. Pekarskaya, and R. S. Karpov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 6, pp. 584-586, June, 1995
Original article submitted June 16, 1994

Levels of lipoperoxides and hydrophobic scavengers of free radicals were estimated in n-hexane extracts of myocardial biopsy specimens taken from cardiac patients (children and adults) during surgical operations. Ultraviolet spectra of these extracts were found to contain four absorption bands with peaks at 214, 233, 258, and 298 nm that characterize the levels of diene and triene conjugates. A comparison of the data obtained for children and adults suggested that the total antiradical activity of the cardiac muscle decreases while its content of vitamin E remains virtually unchanged during ontogeny. Significantly reduced vitamin E levels were noted in specimens from patients with chronic ischemic heart disease.

Key Words: *human heart; age; ischemia; lipid peroxidation; antiradical activity*

The endogenous cellular antioxidant system is a regulator of peroxidation processes that proceed throughout an individual's life [7]. Lipoperoxides and antioxidants participate in the regulation of selective ion transport and in the formation of ion channels in the membrane lipid bilayer [1,2,10,11]. On the other hand, peroxidation is implicated in the pathogenesis of many cell injuries [7]. Experimental work has shown that a variety of naturally occurring or synthetic antioxidants are effective in preventing ischemic and reperfusion-associated damage to cardiac muscle cells [5]. Beneficial effects of antioxidant therapy have also been reported from clinical studies of patients with heart disease [3]. However, many aspects of antioxidant therapy, especially in chronic conditions, remain unexplored and, moreover, antioxidants are not always helpful [3]. One reason for the low efficacy of such therapy may be that we know too little about how the intrinsic antiradical system of the human myocardium is organized. In earlier studies, the system of lipid-soluble antioxidants was assessed for the rat myocardium [11], whole human blood [9], and formed elements of human blood [2,6,9]. The aims of the present study were to estimate lipoperoxide levels and evaluate the system of hydrophobic free radical scavengers (HFRS) in the human myocardium.

MATERIALS AND METHODS

The material used in this study comprised atrial biopsy specimens taken from patients during surgical operations. Biopsy specimens from the following patient groups were examined: children aged 1.5-7 years with congenital heart disease (group 1); patients with an impaired conduction system

*Research Institute of Experimental Cardiology, Cardiology Research Center, Russian Academy of Medical Sciences, Moscow; Research Institute of Cardiology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences

0007-4888/95/0006-0561$12.50

Table 1. Lipoperoxide Levels (Optical Density Units per mg Wet Tissue Weight) in Hydrophobic Extracts of Human Myocardial Biopsy Specimens

Patient group	Absorption band, nm			
	298	258	233	2141
1	2.80±0.16 (4)	6.80±1.92 (7)	9.92±3.84 (4)	48.32±13.12 (4)
2	5.44±0.88 (5)	5.12±1.68 (6)	41.44±12.24 (4)	30.64±7.12 (4)
3	3.52±1.12 (7)	7.52±1.60 (19)	29.44±7.92 (5)	54.88±10.16 (14)
4	10.8±0.56 (4)	15.6±5.2 (4)	0 (7)	54.64±11.68 (6)

Note. n = number of specimens.

(VPW syndrome) (group 2); patients with ischemic heart disease (IHD) in which an aortocoronary bypass was indicated (group 3); and IHD patients with indications for cardiomyoplasty (group 4). The patients of groups 2-4 ranged in age from 37 to 56. Biopsy material was rapidly washed free of blood and frozen in liquid nitrogen.

Hydrophobic myocardial antioxidants were extracted at 4°C. For this, 800 μl of a 50 mM aqueous solution of disodium edetate (Na_2-EDTA) was added to 100 mg of biopsy material, and the mixture was ground in a mortar at the temperature of liquid nitrogen and placed in a glass test tube. After the addition of 2 ml of 96% ethanol, the test tube was vigorously shaken in a vortex mixer for 15 sec; 4 ml of n-hexane were then added and the nonpolar fraction containing lipid-soluble myocardial antioxidants was extracted by homogenization (for 15 sec) in a polytron (Polytron Brinkman Instruments). After the phases underwent spontaneous separation, the upper hexane layer was collected and 4 ml of n-hexane were added to each test tube with samples, which were then homogenized again to achieve further extraction. For the analysis, the first and second extracts were pooled. In each sample, levels of lipoperoxides (diene and triene conjugates) and of HFRS, including vitamin E, were estimated. Lipoperoxides were estimated from the ultraviolet absorption spectra of the extracts, while vitamin E was measured fluorimetrically [12] at an emission wavelength of 322 nm arising upon excitation of fluorescence with monochromatic light of 295 nm. The pool of active lipid-soluble free radical scavengers was evaluated from the degree of bleaching undergone by the n-hexane solution of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (50 μmol/liter) (Sigma), by measuring light absorption at 508 nm using a modification [11] of the Blois method [4]. For determination of the total HFRS pool (which included both the active and activated components), a freshly prepared hydrogen-releasing 1% aqueous solution of sodium borohydride was added to the n-hexane solution. The biphasic system was shaken for 15 sec; 10 min after separation of the phases, the upper hexane layer was collected for analysis.

Magnitudes of the free radical scavenger pools were expressed in tocopherol equivalents using calibration curves plotted for standard α-tocopherol (Sigma) solutions, and the values obtained were normalized per gram of wet myocardial tissue.

RESULTS

Ultraviolet spectra of the n-hexane extracts from the myocardia were found to contain four absorption bands with peaks at 214, 233, 258, and 298 nm (Table 1). The most conspicuous bands were peaking at 214±1 and 258±1 nm. The 233±1 nm band was recorded in 37% of the biopsy specimens from group 1 (n=8), 40% of those from group 2 (n=10), 29% of those from group 3 (n=21), and in none from group 4 (n=7), which consisted of patients with an extreme form of chronic IHD.

Table 2. Effects of Age and IHD on Levels of Vitamin E and Other Hydrophobic Antiradical Agents (nmol Equivalents of Vitamin E per g of Wet Tissue) in Human Myocardial Biopsy Specimens

Antioxidants	Patient group			
	1	2	3	4
Total pool	205.0±15.0*	124.0±9.4*	107.7±5.6	85.0±2.5*
Active	162.0±11.3*	109.0±5.8	106.8±4.4	96.5±1.0
Vitamin E	110.0±29.0	95.0±21.0	95.2±10.0	58.5±14.5*

Note. *p<0.05 relative to group 3.

Since the short-wave 214 nm band characterizes the content of fatty-acid residues in the lipid extracts, it may be concluded that the levels of these lipids did not depend on the patients' age (groups 1 and 2) or IHD severity (groups 3 and 4).

The 233 nm and 258 nm bands reflect the levels of, respectively, diene and triene conjugates in the myocardial lipid fraction [13]. The content of diene conjugates proved to be markedly age-dependent; their levels were 9.9 optical density (OD) units per mg wet weight in group 1 (children) and 41.4 OD units/mg wet weight in group 2 (adults). In group 3 (patients with IHD), diene conjugates were present at lower levels than in group 2 (patients of the same age without IHD). In group 4 (patients with IHD in its extreme form), no spectral band corresponding to diene conjugates could be registered. In the biopsy specimens from this group, 258 nm and 298 nm bands predominated (15.6 and 10.8 OD units/mg wet weight, respectively - Table 1) and the level of triene conjugates (258 nm band) was at least twice as high as in the other groups ($p<0.05$). The data in Table 1 indicate that polyunsaturated fatty acids are particularly susceptible to peroxidation in IHD, with the result that triene conjugates accumulate [8,13]. A similar effect is to be expected for patients in whom antiradical activity of the myocardium itself is lowered.

The membrane antioxidant system is represented by vitamin E and other lipid-soluble free radical scavengers [2,9,11], and their quantitative estimates are presented in Table 2. Comparison of the estimates for children and adults (groups 1 and 2) suggests that the level of this vitamin remains virtually unchanged during ontogeny. A significant fall in vitamin E was only recorded among patients with the extreme form of IHD (group 4). The levels of active free radical scavengers were found to exceed those of vitamin E by factors of 1.1 to 1.6. In terms of decreasing HFRS levels in the biopsy specimens from the four patient groups, these are distributed as follows: 1>2>3>4. Moreover, this distribution also holds for the total HFRS pool as measured in the same myocardial extracts after their treatment with sodium borohydride (Table 2). These findings indicate that vitamin E makes up a substantial proportion of the hydrophobic antioxidant system in the human heart. Vitamin E has been shown to be the major lipid-soluble antioxidant in human plasma [6] and to constitute 50% of the erythrocytic antioxidant system in human blood [9]. A reduction in the total myocardial hydrophobic antioxidant pool during ontogeny, similar to that found in the present study on humans, was noted in a study of antioxidants in the rat myocardium [11].

The lipoperoxide and vitamin E profiles obtained in this study for patients with IHD reaffirm that vitamin E possesses strong antioxidant activity. Other antioxidants, both active and activated, are more sensitive than vitamin E to the conditions under which the cardiac muscle is functioning in IHD. Elucidating the chemical nature of the substances constituting the antioxidant system is essential for understanding the intergroup differences observed in this study.

This work received financial support from the Russian Foundation for Basic Research (grant № 95-04-13177).

REFERENCES

1. A. V. Lebedev, in: *Itogi nauki i tekhniki. Ser. Biofizika [Recent Advances in Science and Technology. Biophysics Series]*, Vol. 33, Moscow (1990), pp. 1-212.
2. E. V. Negresku, A. V. Lebedev, G. N. Baldenkov, *et al.*, *Vopr. Med. Khimii*, **38**, № 1, 36 (1992).
3. J. G. Bieri, *New Engl. J. Med.*, **308**, 1063 (1983).
4. M. S. Blois, *Nature*, **181**, № 4617, 1199 (1958).
5. R. Bolli, M. O. Jeroudi, B. S. Patel, *et al.*, *Circulat. Res.*, **65**, 607 (1989).
6. G. W. Burton, A. Jouce, and K. U. Ingold, *Arch. Biochem. Biophys.*, **221**, № 1, 281 (1983).
7. B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford (1989), pp. 1-540.
8. A. E. Holley, M. K. Walker, K. H. Cheeseman, and T. F. Slater, *Free Radic. Biol. Med.*, **15**, 281 (1993).
9. A. V. Lebedev, *Excerpta Medica (Amsterdam)*, **998**, 601 (1992).
10. A. V. Lebedev and D. O. Levitsky, in: *CRC Handbook of Free Radicals and Antioxidants in Biomedicine*, Eds. J. Miguel *et al.*, Boca Raton (1989), p. 117.
11. A. V. Lebedev, S. M. Sadretdinov, V. Pelouch, *et al.*, *Biomed. Biochim. Acta*, **8**, № 2-3, S122 (1989).
12. J. L. Marx, *Science*, **235**, 529 (1987).
13. T. F. Slater, *Methods Enzymol.*, **105**, 283 (1984).