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ENZYMATIC UTILIZATION OF LIPID PEROXIDES IN THE ATHEROSCLEROTIC HUMAN AORTA

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UDC 616.132-004.6-07:616.132-008.931:
577.152.193

KEY WORDS: glutathione-S-transferases; glutathione peroxidase; atherosclerosis.

The intensity of lipid peroxidation (LPO) in the body changes in certain pathological states associated with disturbance of lipid metabolism [1]. Hyperlipidemias, accompanying the development of atherosclerosis, also are characterized by an increased velocity of LPO. In particular, a marked increase in the concentration of lipid peroxides is found in patients with atherosclerosis in the blood [2] and in zones of atherosclerotic lesions in the aorta [6]. It is suggested that elevation of the lipid peroxide level is one cause of the development of atherosclerosis because of the ability of hydroperoxides to cause damage to the intima of blood vessels [14], to stimulate platelet aggregation and proliferation of smooth-muscle cells [12], and also to inhibit prostacycline synthesis in the vascular wall [7]. An important role in the detoxication of lipid peroxides in the body is played by glutathione peroxidase (GP) and glutathione-S-transferases (GT) [9, 5]. The latter differ from GP in their ability to reduce not only hydroperoxides of free fatty acids, but also the aliphatic acyl groups of membrane phospholipids [5].

Since no systematic investigations of changes in GT activity in atherosclerosis have been undertaken, the aim of the present investigation was to study GT activity in specimens of the intima and media of the human aorta infected with different degrees of atherosclerosis.

EXPERIMENTAL METHOD

The thoracic aortas of persons aged from 27 to 72 years, dying from cardiovascular diseases, were taken for investigation. Autopsies were performed in the A. L. Myasnikov Institute of Clinical Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, 1-6 h after the stated time of death. The intima and media from the intact zone of the vessel, and the regions of lipid stains and lipid-fibrous plaques were separated mechanically, after which the tissue samples obtained were homogenized in 50 mM phosphate buffer, pH 7.4 (1:5), the homogenate was centrifuged for 10 min at 800g, and the supernatant was frozen in liquid nitrogen and kept at −20°C. GT activity was determined with 1-chloro-2,4-dinitrobenzene (CONB) and with p-nitrophenyl acetate (NPA) as described previously [13]. Determination of activity with 1-fluoro-2,4-dinitrobenzene (FDNB) was carried out under standard conditions [13] with addition of the substrates (glutathione and FDNB) in a concentration of 0.1 mM. The unit of GT activity was taken to be the quantity of enzyme catalyzing the formation of 1 μmole of product during 1 min under the conditions of determination.

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Department of Human Cardiovascular Pathology, A. L. Myasnikov Institute of Clinical Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 10, pp. 444-446, October, 1988. Original article submitted October 18, 1987.

TABLE 1. GT Activity and Peroxidase Activity (in U/g protein) in Atherosclerotic Zones of the Human Thoracic Aorta (M ± m)

Test object	Substrate						
	CDNB	FDNB	NPA	NG	TBHP	CHP	H_2O_2
Intima:							
undamaged	93,8±13,0 (27)	47,5±5,8 (27)	4,15±0,64 (26)	0,92±0,09 (27)	12,5±1,8 (26)	7,5±1,4 (27)	7,0±1,7 (27)
lipid stain	101,4±14,9 (23)	44,9±6,3 (23)	5,59±0,74 (23)	0,63±0,06* (23)	18,3±2,6 (23)	9,2±1,8 (23)	10,1±1,8 (23)
lipid-fibrous plaque	46,5±7,6* ** (22)	29,0±7,0* (22)	3,69±0,37** (22)	0,85±0,14 (22)	18,7±3,8 (22)	8,9±2,3 (22)	13,7±2,9* (22)
Media:							
undamaged	251,9±31,5 (27)	70,5±5,8 (27)	7,43±1,05 (27)	0,93±0,11 (27)	8,1±1,1 (27)	4,3±0,5 (27)	5,6±0,9 (26)
lipid stain	295,4±46,9 (23)	77,0±7,3 (23)	7,9±1,05 (23)	0,97±0,14 (23)	11,2±1,7 (23)	5,6±0,8 (23)	6,8±1,1 (23)
lipid-fibrous plaque	188,4±25,7** (20)	63,8±9,7 (20)	5,61±0 60** (20)	0,89±0,14 (20)	12,7±3,0 (20)	6,5±2,3 (20)	9,9±1,6* (20)

Legend. Number of specimens tested given in parentheses. *$p < 0.05$ compared with unaffected tissue, **$p < 0.05$ compared with tissue from region of lipid stain.

The nitrite reductase and peroxidase activity of GT was determined by the use of nitroglycerine (NG), tert-butyl hydroperoxide (TBHP), and cumene hydroperoxide (CHP) respectively, measuring activity of the enzyme with respect to NADPH oxidation in a coupled glutathione reductase system, as described previously [3]. Activity of selenium GP was measured by the use of H_2O_2 as the substrate [3]. The unit of activity of GT and GP when the coupled GSSG-reductase system was used was taken to be the quantity of enzyme required to oxidize 1 μmole NADPH in 1 min. The measurements were made on a "Labsystems Oy FP-901" chemical analyzer (Finland) on the kinetic mode. Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS

We know from the literature that CDNB is a good substrate for all types of GT, although the highest reaction velocity was found for neutral and acid isozymes [10]. Organic hydroperoxides are preferential substrates for alkaline GT, whereas neutral transferases react only weakly with them, and acid transferases virtually not at all [10, 13]. We also know that H_2O_2 is not a substrate for GT [11], but it is a good substrate for selenium GP, which reacts also with organic hydroperoxides [10].

The experimental results, given in Table 1, show that GT activity in the undamaged media of the human aorta is significantly higher than in the intima: by 2.7, 1.5, and 1.8 times ($p < 0.05$) when CDNB, FDNB, and NPA respectively are used as substrates, whereas the lipid peroxidase activity (activity of the enzyme when organic hydroperoxides are used as substrates), on the other hand, was higher in the undamaged intima when in the undamaged media ($p < 0.05$). Since selenium GP activity (substrate — H_2O_2) is virtually identical in the intima and media, differences in lipid peroxidase activity levels in the intima and media must be due rather to differences in the content of GT isozymes. Since the transferase activity of GT in the media is higher, it can be tentatively suggested that the intima is richer and the media poorer in alkaline forms of GT, which possess high lipid peroxidase activity.

GT and lipid peroxidase activity in the zone of the lipid stains in the human aorta are virtually indistinguishable from those in the undamaged intima and media of the aorta (Table 1). Meanwhile GT activity in the intima from the region of a lipid-fibrous plaque is lower than in the undamaged intima.

Lipid peroxidase activity is unchanged in zones of atherosclerotic damage to the intima and media, although there is a tendency toward an increase compared with that observed in the undamaged intima and media of the vessels. An increase in peroxidase activity (selenium GP) was observed in the intima and media from zones of lipid-fibrous plaques compared with this parameter in the unaffected region.

Data published previously are evidence of reduction of lipid peroxidase activity (when TBHP was used as substrate) in zones of atherosclerotic lesions in the human aorta [9]. The

following explanation of the contradiction observed between data cited [9] and obtained in the present investigation may be suggested. Lipid peroxidase activity is determined by the action of selenium (GP) and nonselenium (GT) peroxidases. Since changes in the peroxidase activity of GP and GT during progressive atherosclerosis are opposite in direction and may compensate one another, differences in total lipid peroxidase activity between the normal and affected regions may prove to be not significant.

The data given in this paper are thus evidence that lipid peroxidase activity in zones of atherosclerotic damage to the human aorta may arise through a decrease in GT activity. This is a very important fact for the pathogenesis of atherosclerosis, having regard to the important role of GT in detoxication of membrane acyl hydroperoxides, the content of which rises sharply in the course of progressive atherosclerosis of the aorta [7].

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BIOCHEMICAL AND IMMUNOCHEMICAL STUDY OF STRUCTURAL HETEROGENEITY OF POLYMERIZED HEMOGLOBIN

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UDC 615.385.16.074

KEY WORDS: polymerized hemoglobin; heterogeneity; molecular weight; immunochemical properties.

The use of polymerized hemoglobin (Hb), containing pyridoxal-5'-phosphate as regulator of reversible oxygenation (PH-PP) is evidently the most promising approach to the creation of a blood substitute with a gas transporting function [1, 4, 5]. Experiments in animals have shown that PH-PP circulates for a long time in the blood stream, has a half-elimination time of 25-48 h, transports virtually sufficient oxygen to the erythrocytes, and maintains the survival of animals after replacement of 95% of the circulating blood volume [2, 7, 8]. During

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(Presented by Academician of the Academy of Medical Sciences of the USSR O. K. Gavrilov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 106, No. 10, pp. 446-448, October, 1988. Original article submitted July 21, 1987.