BLOOD GLUTATHIONE PEROXIDASE II ACTIVITY IN MAMMALS WITH HYPERCHOLESTEREMIA

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According to Harman's hypothesis [9], an increase in the concentrations of lipid peroxidation products (LPP) in the blood and aorta during atherogenesis [4, 6, 7] leads to disturbance of metabolism, permeability of the vessel wall, and to its injury through the formation of stable lipid—protein polymer complexes [11], oxidation of protein SH groups, and changes in the activity of certain enzymes [1, 4, 5]. Disturbance of metabolism of lipid peroxides may thus be an important step in the pathogenesis of atherosclerosis [4].

Since hyperlipoproteinemia and hypercholesteremia are among the "risk factors" in the onset of atherosclerosis [6], it was decided to study the activity of glutathione peroxidase II (glutathione: organic hydroperoxide oxidoreductase), an enzyme detoxicating lipid peroxides [8], in the blood of mammals differing in their susceptibility to atherosclerosis (under normal conditions and during hypercholesteremia).

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 180 ± 20 g were given a semisynthetic atherogenic diet (60.7% starch, 22% casein, 10% plum oil, 4% mixed salt, 3% cellulose, 0.2% choline chloride, 0.1% of a mixture of water-soluble vitamins in glucose), containing 0.5% deoxycholic acid and 0.5% cholesterol, for four weeks. Male chinchilla rabbits weighing 2.2 ± 0.2 kg were given 0.2 g cholesterol/kg body weight daily by gastric tube for 12 weeks, in the form of a suspension in sunflower oil. Mini-pigs (male) from the Breeding Group, Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, weighing 55 ± 5 kg, received 1 g cholesterol/kg body weight daily for two weeks with their basic diet. Investigation of patients (men aged 40-55 years) with ischemic heart disease (IHD), due to atherosclerosis of the coronary arteries (as shown by selective coronary angiography), was carried out during the investigative period of their stay in the clinic of Institute of Cardiology, All-Union Cardiologic Scientific Center (ACSC), Academy of Medical Sciences of the USSR (the patients received no drugs). Animals of control groups were kept on the corresponding diet but without the addition of cholesterol. The human control group consisted of men aged 40-49 years without hyperlipidemia or clinical manifestations of IHD, chosen during an epidemiological investigation at ACSC, Academy of Medical Sciences of the USSR. Glutathione peroxidase (GP) II activity in samples of whole blood was determined after hemolysis with Triton X-100 by the method described previously, using tert-butylhydroperoxide as the substrate [2]. The presence of large amounts of hemoglobin did not interfere with determination of the activity of this enzyme, for preliminary precipitation of hemoglobin by the method in [10] did not lead to any appreciable increase in the rate of oxidation of NADPH in the coupled glutathione-reductase system. The total plasma cholesterol concentration was determined by the AA-2 automatic analyzer (from Technicon, USA) [3]. The concentration of acyl hydroperoxides was determined spectrophotometrically and the concentration of secondary LPP — intermolecular "cross-linkages" in aminophospholipids — was determined spectrofluorometrically as described previously [3].

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TABLE 1. Blood GP-II Activity in Mammals with Hypercholesteremia (M ± m)

Species	Group	Total cholesterol concentration		GP-II activity	
		mg/ml	%	units/ml	%
Rat	Control (n = 38)	0,63±0,02	100	20,40±2,76	100
	Experiment (n = 30)	1,24±0,1	197†	19,10±2,35	94
Rabbit	Control (n = 17)	0,33±0,035	100	4,30±0,19	100
	Experiment (n = 19)	2,44±0,44	739†	2,60±0,63	61†
Mini-pig	Control (n = 7)	0,46±0,02	100	4,50±0,18	100
	Experiment (n = 7)	0,85±0,07	185†	2,40±0,29	53†
Man	Control (n = 48)	2,15±0,07	100	2,00±0,11	100
	Patients with coronary atherosclerosis and hypercholesteremia* (n = 11)	3,03±0,06	141†	1,10±0,16	55†

*Blood cholesterol concentration > 276 mg/100 ml
†Differences between values significant at P < 0.05 level.

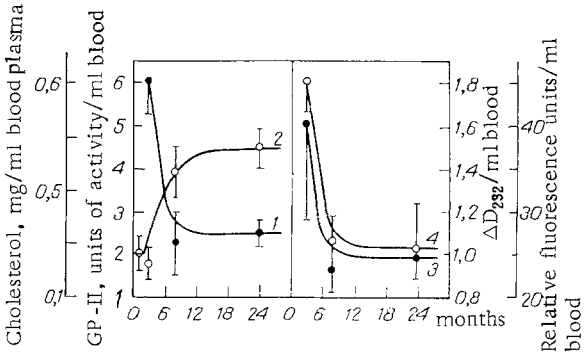

Fig. 1. Changes in cholesterol concentration (1), GP-II activity (2), and in blood levels of primary (3) and secondary (4) LPP in mini-pigs of different ages.

EXPERIMENTAL RESULTS

The principal results are summarized in Table 1. Comparison of the results of determination of GP-II activity in the blood of the control animals shows that it was considerably higher in rats, which are resistant to the development of atherosclerosis, than in rabbits and mini-pigs, which are susceptible. GP-II activity in human blood of the control group was significantly lower than in the blood of any of the species of laboratory animals tested. So far as the level of activity of protective enzyme systems is concerned, by virtue of his special biological features, man is thus a species with comparatively little protection against the harmful action of lipid peroxides.

Alimentary hypercholesteremia leads to a marked decrease in GP-II activity in the blood of mammals susceptible to atherosclerosis (rabbits, mini-pigs), whereas in the species resistant to the development of atherosclerosis (rats) the blood activity of this enzyme was unchanged despite an almost twofold increase in the cholesterol concentration. In patients with coronary atherosclerosis and hypercholesteremia, blood GP-II activity also was significantly lower than in the control (Table 1).

GP-II activity during hypercholesteremia was reduced not only in whole blood, but both in the plasma and the blood cells. For instance, it was lowered in the plasma and erythrocytes of the experimental rabbits by 1.9 (n = 22, P < 0.001) and by 1.7 (n = 17, P < 0.05) times respectively, and in the platelets of the experimental mini-pigs by 1.4 times (n = 7, P < 0.05).

To determine whether GP-II activity changes when the blood cholesterol concentration falls, the activity of this enzyme was studied in the blood of mini-pigs of different ages. According to data in the literature [12], the blood cholesterol concentration of mini-pigs falls significantly during postnatal development. In full agreement with these facts, the blood cholesterol concentration of the mini-pigs in these experiments fell from the third to the eighth month of life by almost 60%, but thereafter it remained unchanged with age of the animals (Fig. 1, curve 1). The decrease in the blood cholesterol concentration of the mini-pigs was accompanied by a corresponding increase in GP-II activity (Fig. 1, curve 2); at the same time a decrease was observed in the concentrations of primary (acyl hydroperoxides) and secondary (intermolecular "cross-linkages" in aminophospholipids) LPP (Fig. 1, curves 3 and 4). These results confirm the existence of definite correlation between the concentration of LPP and GP-II activity in the blood [4, 5]; it was also found, moreover, that the blood cholesterol level has a marked effect on activity of the enzyme (Fig. 1, Table 1). The influence of hypercholesteremia on GP-II activity, it should be noted, is manifested only in species of laboratory animals susceptible to atherosclerosis and in man (Table 1). The resistance of rats to development of atherosclerosis can be explained by the high level of GP-II activity and its considerable resistance to the action of hypercholesteremia, which must prevent the accumulation of toxic lipid peroxidation products in the tissues of these animals. No correlation could be found between the severity of the hypercholesteremia and the degree of lowering of GP-II activity in rabbits and mini-pigs (the initial GP-II activity and initial blood cholesterol level of these species, as the results in Table 1 show, are similar). This fact may be explained on the grounds that to produce the greatest fall in GP-II activity (where this occurs) an increase in the normal blood cholesterol by 1.5-2 times was sufficient (Table 1). Despite the fact that the concrete mechanism of action of hypercholesteremia on GP-II activity is unknown, the biological role of this newly discovered phenomenon appears to be very important from the standpoint of the possible participation of lipid peroxides in the development of atherosclerosis.

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