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EFFECT OF UNITHIOL AND ACETYLCYSTEINE ON LIPID PEROXIDATION AND THE ERYTHROCYTE ANTIOXIDANT SYSTEM OF SENSITIZED GUINEA PIGS

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An important place in the response of the body to foreign proteins is occupied by changes in the redox balance and antioxidant activity of the tissues [1, 7], which lead to a disturbance of membrane structure and of cell metabolism. These disturbances are manifested clearly in erythrocytes which, in the course of immunoallergic processes, are exposed to the action of antigens, immune complexes, and free radicals [2, 6]. According to the observations of several workers, modification of erythrocyte membranes in pathological processes reflects changes taking place in the plasma membranes of other cells [3], and for that reason erythrocytes can serve as a model with which to study the cellular resistance of the body as a whole. A responsible role in the resistance of the cells to the action of harmful factors is played by low-molecular-weight thiols and, in particular, by glutathione, and also by enzymes participating in its reduction [11, 12]. The thiols can exert a direct action on the reactions of immunity [8] and they are components of immunocorrective agents [5]. There is evidence that acetylcysteine influences allergic reactions in man and animals [15].

In connection with the development of the microbiological production of forage proteins the probability of human development of hypersensitivity to yeast-like fungi is increased, and for that reason the study of the mechanism of action of thiols in experimental sensitization is an urgent problem, and the investigation described below was undertaken in order to study it.

EXPERIMENTAL METHOD

Experiments were carried out on guinea pigs of both sexes weighing 400-450 g. The animals were divided into two equal groups. Animals of group 1 were sensitized by a single subcutaneous injection of a suspension of a heat-killed and dried culture of *Candida maltosa* in physiological saline, equivalent to 30 mg/kg body weight. Animals of group 2 were given an equal volume of physiological saline. Each group was divided into three subgroups, each consisting of six animals. Guinea pigs of the first and second subgroups were given intra-

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TABLE 1. Biochemical Changes in Erythrocytes of Sensitized Guinea Pigs Exposed to the Action of Thiol Antioxidants (M ± m)

Parameters studied	Sensitization			Control		
	unithiol	acetylcysteine	physiological saline	unithiol	acetylcysteine	physiological saline
Area of skin reaction, mm^2	75* (61—108)	61* (27—108)	75* (25—108)	36 (19—75)	19 (12—61)	12 (7—36)
Ratio of areas of skin reaction in sensitized and control animals	2,1	3,2	6,2			
Erythrocyte resistance, hemolysis, %	96,0±10,0*	78,0±9,5*	85,0±8,0*	69,0±6,0	91,6±11,5	52,0±6,5
LPO, mmoles malonic dialdehyde/liter	20,82±1,23	20,00±1,23	21,62±1,70*	20,23±0,66	18,87±1,47	17,85±1,40
Thiol-disulfide ratio:						
Protein	2,81±0,37	3,29±0,36	3,24±0,34	2,79±0,43	2,95±0,40	3,05±0,34
Nonprotein	2,50±0,16	2,72±0,32	2,08±0,34*	3,20±0,44	2,30±0,33	3,20±0,43
G6PDH activity, ncat/g	22,50±1,63	36,00±3,60	48,70±6,00	38,15±6,30	42,20±3,88	53,50±14,00
GR activity, ncat/g	6,62±0,83*	8,18±0,98	6,07±1,28*	7,38±1,37	8,23±1,08	9,42±2,10
LDH activity, ncat/g	161,0±21,8*	276,0±26,5	207,9±27,7*	280,0±48,0	195,0±25,6	391,0±13,0

Legend. *) Statistically significant changes in sensitized animals compared with controls.

peritoneal injections of a solution (0.5%) of unithiol and acetylcysteine in a dose of 5 mg/kg once daily. The first injection was given on the day before sensitization, the last seven injections during the 10 days after the end of sensitization. Animals of the third subgroup received, instead of unithiol and acetylcysteine, equal volumes of physiological saline at the same times. All animals were given an intradermal injection of 0.1 ml of *C. maltosa* autolysate 10 days after sensitization, with a protein content of 100 µg/ml. A further 2 days later the animals were anesthetized with ether and decapitated. The state of sensitization was determined by the presence of a skin reaction and by the level of antibodies detectable by the immunofluorescence test, conducted by the indirect method [14]. Lipid peroxidation (LPO) was determined by the reaction with 2-thiobarbituric acid. Activity of glucose-6-phosphate dehydrogenase (G6PDH), gluthathione reductase (GR), and lactate dehydrogenase (LDH) was determined spectrophotometrically [4, 9] and the protein content in the samples was measured by Lowry's method. The content of sulfhydryl groups and disulfide bonds was determined by amperometric titration [10]. The method of hemolysis with urea [3] was used as an integral test, characterizing erythrocyte membrane resistance. The significance of differences between the skin reactions was estimated by the Wilcoxon—Mann—Whitney nonparametric method, and the significance of differences between the other parameters by Student's test.

EXPERIMENTAL RESULTS

The skin test on the sensitized animals was most marked after 24 h, after which it diminished. The results of the skin test 24 h after injection of the yeast autolysate are given in Table 1. A tendency for the skin reaction to strengthen was observed in the control animals receiving the antioxidants. In sensitized animals receiving and not receiving the antixoidants, there was no difference in the areas of the pupules. However, judging by the ratio between the areas of the skin tests in analogous subgroups in sensitized and control animals, when unithiol and acetylcysteine were used the specific reactions to the allergen were weaker than in animals receiving physiological saline. This phenomenon can be explained by the effect of thiols on the immune response and, in particular, by inhibition of LPO [13]. The titer of specific antibodies in the sera of the different groups and subgroups of animals did not differ and its value was between 1/2 and 1/32, which, if compared with the results of the skin test, is evidence that the experimental animals had developed sensitization resembling the Arthus reaction in type. Increased hemolysis of erythrocytes was observed in the sensitized animals not receiving the antioxidants, when the urea concentration was 165 mM, their LPO was activated, their nonprotein thiol-disulfide ratio was lowered, and their LDH and GR activity also was depressed. After injection of unithiol and acetylcysteine into the control animals no statistically significant changes were observed in the parameters tested. In sensitized animals receiving unithiol, the decrease in resistance of the erythrocytes and also in LDH and GR activity was significant compared with the control.

No statistically significant differences were found between the biochemical parameters in the different groups of animals receiving acetylcysteine. The results indicate that structural changes in the erythrocyte membranes during sensitization are closely linked with depression of thiol-disulfide equilibrium and with modification of the enzymes. Under the conditions used, thiol antioxidants were able to depress LPO and to normalize the thiol-disulfide balance in the sensitized animals. However, enzyme activity and erythrocyte resistance were partially restored only by the action of acetylcysteine, evidently due to the higher degree of oxidizability of unithiol compared with acetylcysteine and the greatest hydrophobicity of the latter. The stable tendency of G6PDH activity to decline in the animals under the experimental conditions will be noted, and a further study of the dose-dependent effects of the thiol antioxidants is called for during sensitization.

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ANTIOXIDANT EFFECT OF HIGH-DENSITY LIPOPROTEINS IN PEROXIDATION OF LOW-DENSITY LIPOPROTEINS

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Increased sensitivity of low-density lipoproteins (LDL) to peroxidation *in vitro* under the influence of oxygen and Fe^{++} [6, 14], and of long-term dialysis in medium not containing antioxidants [15], are well-known facts. There is also evidence of peroxide modification of LDL *in vivo* in atherosclerotic plaques [1]. LDL isolated from blood plasma in the presence of antioxidants nevertheless contain large quantities of diene conjugates and fluorescent Schiff bases [15].

One result of peroxidation of polyene acyl groups of phospholipids, composing LDL, is that they acquire new properties: a change in the conformation of apoprotein B, the basic protein of LDL, which may be the cause of the appearance of antigenicity among them [5];

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