Age-Related Differences in the Degree of Lipid Peroxidation and State of Antioxidant Protection under the Influence of Alloxan

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Alloxan-induced hyperglycemia was accompanied by activation of lipid peroxidation processes with different degree of their completeness depending on the age of experimental animals. Lipid peroxidation in young animals mainly resulted in the formation of end-products, while in adult animals the initial stage of oxidation prevailed. Alloxan inhibited the enzymatic and nonenzymatic systems of antioxidant protection, which impaired adaptation and caused oxidative stress.

Key Words: *age-related differences; alloxan; lipid peroxidation; antioxidant protection; oxidative stress*

Studying the prophylactic effect of natural and synthetic substances often involves experimental modeling of pre-disease. Metabolic changes in the organism are insignificant and reversible under these conditions. Administration of alloxan in single doses of 40-180 μg/kg caused diabetes mellitus in rats [1,5,11]. There are schemes for the treatment with alloxan that cause various pathological states (pre-diabetes or impaired glucose tolerance) [4]. Changes in the degree of lipid peroxidation (LPO) and state of the antioxidant system (AOS) under these conditions received little attention. Age-related differences in the severity of oxidative stress remain unknown. Here we studied age-related metabolic differences in the LPO—AOS system during alloxan-produced pre-diabetes.

MATERIALS AND METHODS

Experiments were performed on 54 male Wistar rats aging 2-2.5 (young animals) and 6 months (adult animals). The animals fed a standard diet and had free access to water. The rats were decapitated under ether anesthesia; the blood and liver were obtained for the analysis. The rats of each age group were divided into 2 subgroups: intact animals and rats receiving intramuscular injections of alloxan in increasing doses of 40, 60, and 80 mg/kg at a 7-day interval.

The degree of LPO was estimated by the content of conjugated dienes, malonic dialdehyde (MDA), and Schiff bases (SB) in erythrocyte hemolysate and liver homogenate [3,9,12]. The system of antioxidant protection (AOP) was assayed by the integral index of plasma antioxidant activity (AOA), amount of reduced glutathione, activities of glutathione reductase and glutathione peroxidase, and contents of α-tocopherol and vitamin A in the blood and liver [8,9]. The results were analyzed by methods of variational statistics on IBM PC. The significance of differences was estimated by Student's *t* test.

RESULTS

The severity of alloxan-produced hyperglycemia and microalbuminuria differed in young and adult rats. Blood glucose level and urine albumin concentration

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in young males increased by 17.8 (2.93±0.04 mmol/liter) and 15.4% (4.70±0.02 mg/day), respectively, compared to intact rats (2.29±0.05 mmol/liter and 3.80±0.04 mg/day, respectively). Blood glucose in adult males was 2.66±0.06 mmol/liter, which reflects the development of pre-diabetes. Administration of alloxan induced not only hyperglycemia, but also moderate hyperlipidemia. It was associated with impairment of glycolysis and activation free radical lipid oxidation. Lipid peroxide concentration increased in rats with alloxan-produced diabetes. These compounds inhibit a key enzyme of cholesterol catabolism (7-α-hydroxylase), which maintains high level of plasma cholesterol and contributes to the development of hyperlipidemia [1,6,7].

Hyperglycemia and lipid disorders in animals are directly related to intensification of free radical lipid oxidation and accumulation of LPO products in the liver and blood, which contributes to the development of disturbances in lipid metabolism (Table 1). The degree of lipid oxidation in the liver is highest in young animals. The contents of conjugated dienes, MDA, and SB in these rats were much higher than in intact animals (by 80, 63, and 31%, respectively). Alloxan increased blood content of conjugated dienes, MDA, and SB in these rats by 45, 32, and 51%, respectively, compared to the control ($p<0.01$). Young animals were characterized by activation of LPO with the formation of end-products in the blood and liver. The degree and completeness of oxidation are determined by the ratio between relative increase in the concentration of conjugated dienes and total content of MDA and SB. The formation of primary products was the major stage of LPO in adult animals. It was confirmed by accumulation of conjugated dienes (compared to other LPO products) and changes in their ratio in the liver and blood of experimental animals. Conjugation of dienes in biological membranes serves as a sensitive test for the formation of lipid hydroperoxides (primary LPO products) [2]. The amount of conjugated dienes in the blood and liver of experimental animals exceeded that in intact rats by 122 ($p<0.01$) and 51% ($p<0.01$), respectively. The concentrations of MDA and SB increased in the liver and especially in the blood of adult rats. In the blood of experimental rats the content of these compounds was 1.5-fold higher than in intact animals. The concentrations of MDA and SB in the liver of experimental rats were higher than in intact animals by 18 ($p<0.05$) and 31% ($p<0.01$), respectively.

The existence of age-related differences in stimulation of LPO was probably accompanied by changes in the metabolism of thiobarbituric acid-reactive substances (MDA and homologous compounds) [10]. Further metabolism of MDA in the liver and blood erythrocytes depended on animal age. Transformation of MDA in erythrocytes from young rats resulted in the formation of SB-like compounds. In the liver of these animals MDA undergoes conversion into malonic acid, which plays a role in the synthesis of higher fatty acids. Changes in MDA catabolism were similar in adult rats. However, further transformation of MDA in the liver and blood of these animals mainly resulted in the formation of SB.

Alloxan-induced LPO activation increased consumption of endogenous antioxidants in the liver of animals, which was accompanied by a decrease in the concentration of these compounds (compared to that in intact rats). The nonenzymatic AOP system was characterized by a significant decrease in the concentrations of α-tocopherol and retinol in the liver of young (by 17 and 18%, respectively) and old animals (by 23 and 19%, respectively). Blood concentration of α-tocopherol in young and old animals decreased by 21 and 24%, respectively ($p<0.001$). After administration of alloxan plasma content of vitamin A (low-molecular-weight antioxidant) decreased in adult rats (by 25%, $p<0.001$), but increased in young animals (by 21%).

The glutathione-dependent enzymatic system of AOP was inhibited in the liver of rats with hyperglycemia. Activities of glutathione peroxidase and glutathione reductase tended to decrease under these conditions. The inhibition of glutathione reductase and glutathione peroxidase plays a similar role, since reduction of lipid peroxides does not proceed without bioregeneration of glutathione [5]. Glutathione peroxidase catalyzes reduction of lipid peroxides by oxidizing glutathione. Glutathione concentration is a rate-limiting factor for this intracellular process. The decrease in the concentration of glutathione is accompanied by activation of LPO [3]. Inactivation of enzymes contributes to the decrease in reduced glutathione concentration in the liver of pre-diabetic animals (compared to intact rats). Suppression of the glutathione-dependent enzymatic system of AOP depended on animal age. Significant changes were revealed in the liver of adult animals. Activity of AOS tended to decrease in young rats. The mechanisms of AOP in these animals were realized only via the enzymatic system, while the content of reduced glutathione remained unchanged. Liver content of reduced glutathione and activities of glutathione peroxidase and glutathione reductase in adult rats were lower by 10 ($p<0.001$), 29, and 23% ($p<0.001$), respectively, than in intact animals. Glutathione reductase activity in young animals decreased by 32%.

The observed changes in AOS upon activation of LPO can be considered as an adaptive response of the organism (*e.g.*, cells and blood erythrocytes). A certain

TABLE 1. LPO—AOP System in the Blood and Liver of Animals from Different Age Groups Receiving Alloxan (*M*±*m*)

Parameter	Young		Adult	
	intact animals (*n*=17)	treated animals (*n*=10)	intact animals (*n*=17)	treated animals (*n*=10)
CD, nmol/mg lipids	2.64±0.02	3.84±0.08***	2.14±0.07	4.75±0.07***
	4.27±0.11	7.69±0.15***	3.71±0.24	5.62±0.12***
MDA, μmol/g Hb	29.23±0.49	38.66±0.50***	31.50±0.93	48.66±0.54***
nmol/mg protein	3.17±0.07	5.18±0.04***	2.07±0.06	2.44±0.11**
SB, arb. units/mg lipids	0.47±0.01	0.71±0.01***	0.45±0.02	0.66±0.01***
	11.96±0.07	15.72±0.11***	11.26±0.33	14.78±0.29***
Blood AOA, %	75.14±5.09	51.26±2.84**	43.75±7.08	43.86±5.29
GL, nmol/g Hb	613.08±5.75	813.81±3.19**	304.81±9.63	239.61±8.22**
μg/mg protein	3.92±0.24	3.45±0.05	8.03±0.12	7.21±0.06***
GR, μmol NADPH/mg Hb/min	2.05±0.05	1.27±0.04***	1.87±0.08	1.30±0.02***
nmol NADPH/mg protein/min	2.76±0.04	1.89±0.10***	1.64±0.10	1.89±0.10***
GP, μmol GL/g Hb/min	24.72±0.55	29.15±1.85*	13.28±1.47	15.24±0.91
nmol GL/mg protein/min	1.46±0.05	1.43±0.05	0.91±0.02	0.65±0.03***
α-TP, ng/mg lipids	250.10±8.88	198.26±4.00**	252.88±9.18	191.78±3.38**
	175.00±2.24	145.00±2.89**	233.60±8.13	180.28±3.77**
Vitamin A, ng/mg lipids	13.62±0.10	16.56±0.55***	15.40±0.69	11.57±0.42***
	68.84±0.76	56.28±1.37***	87.04±1.99	70.24±2.10***

Note. CD, conjugated dienes; SB, Schiff bases; GL, glutathione; GR, glutathione reductase; GP, glutathione peroxidase; α-TP, α-tocopherol. Numerator, blood parameters; denominator, liver parameters. *p<0.05, **p<0.01. and ***p<0.001 compared to intact animals.

relationship was found between the degree of LPO and state of AOP to maintain normal function and integrity of these cells. Pathogenic factors (*e.g.*, alloxan injection) stimulate free radical reactions in the organism, which produces a strain of AOS in erythrocytes. This exposure induces various changes in the enzymatic system of AOP.

Alloxan significantly decreased glutathione reductase activity in the blood of young and adult animals (by 38 and 30%, respectively, compared to intact rats). Glutathione peroxidase activity in young and adult rats increased by 18%. Reduced glutathione content decreased in adult rats (by 21%), but increased in young animals (by 33%).

AOS activity in the blood and liver underwent similar changes in adult pre-diabetic rats. We observed a decrease in glutathione reductase activity and reduced glutathione content. Glutathione peroxidase activity remained unchanged in these animals. No changes were revealed in the integral index of blood AOA that reflects total activity of compounds inhibiting free radical oxidation in the blood.

The degree of LPO in treated rats of different groups was higher than in intact animals. It should be emphasized that the completeness of LPO differed in young and adult animals. Moreover, further metabolism of MDA in the blood and liver depended on the age of experimental animals.

LPO in the blood and liver of young rats was most intensive and resulted in the formation of MDA (end-product). However, differences were revealed in the pathways of MDA transformation in the liver and blood of these animals. It should be emphasized that metabolic conversion of MDA in young rats mainly resulted in the formation of malonic acid. However, the initial stage of oxidation prevailed in adult animals and led to accumulation of primary products. These differences in the contents of primary (MDA) and secondary LPO products (SB) suggest that utilization of MDA in the blood and liver of adult rats mainly results in the formation of SB-like compounds.

The state of AOS in animals with hyperglycemia is characterized by suppression of the second-order antioxidant mechanisms. It is accompanied by the increase in consumption of endogenous low-molecular-weight antioxidants in the liver and blood of young and adult animals. The third-order antioxidant mechanisms involved in degradation of lipid peroxides are suppressed during hyperglycemia. The observed changes are associated with function of glutathione peroxidase; activity of this enzyme in rat liver decreased to a different extent.

Our results suggest that administration of alloxan in the same dose induces different metabolic changes which contributing to the development of pre-diabetes in young and adult animals. Age-related differences in alloxan-induced activation of LPO reflect an imbalance in the LPO—AOP system (activation of LPO, inhibition of enzymatic and nonenzymatic AOS) and development of adaptive disorders and oxidative stress.

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