Effect of Maksar on Antioxidant System in Rats with Type IIa Alimentary Hyperlipoproteinemia

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The development of type IIa alimentary hyperlipoproteinemia in rats was accompanied by changes in activity of glutathione-dependent enzymes, exhaustion of the reserves of antioxidant vitamins in the liver and blood, and intensification of lipid peroxidation. The hepatoprotective preparation Maksar obtained from far-eastern plants *Maachia amurensis* and containing natural biological antioxidants normalized blood lipid composition, corrected liver lipidosis, and improved the antioxidant defense system.

Key Words: *type IIa alimentary hyperlipoproteinemia; lipid peroxidation; antioxidant system; natural antioxidants*

The development of type IIa alimentary hyperlipoproteinemia (HLP) is accompanied by intensification of lipid peroxidation (LPO) in hepatocytes, decrease in the activity of antioxidant enzymes in the cytosol of these cells, and oxidative modification of low-density lipoproteins (LDL) that gain atherogenic activity [6]. Blockade of LPO in the liver would contribute to a decrease in the concentration of circulating modified lipoproteins and correction of HLP. Preparations that increase the reserves of the antioxidant system (AOS) hold much promise in this respect.

Here we studied the effects of Maksar obtained from far-eastern plants *Maachia amurensis* on the intensity of LPO and state of AOS in the blood and liver in rats with alimentary HLP. The preparation corrects hypoproteinemia and fatty degeneration of the liver [1]. Maksar contains natural polyphenols (isoflavonoids and stilbenes) determining its antioxidant properties [12].

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 200-250 g. Control animals were kept in a vivarium under standard conditions. Experimental rats received a diet unbalanced in the contents of proteins, carbohydrates, fats, and cholesterol (CH) for 4 weeks [11]. After the development of HLP these rats fed a standard diet. Maksar was minced, thoroughly suspended in distilled water, and administered intragastrically (240 mg/kg) to some animals with HLP 30 min before feeding. The preparation was given for 35 days. The rats were decapitated under ether anesthesia.

Blood lipids were assayed on a COBAS-MIRA biochemical analyzer. The amount of CH in LDL and very-low-density lipoproteins (VLDL) was calculated by the formula of Friedewald: VLDL CH=triglycerides×0.45 and LDL CH=total CH-(HDL CH+VLDL CH). Atherogenic activity of the blood was estimated by calculating the index of atherogenicity: (total CH-HDL CH)/HDL CH. The intensity of LPO was determined by the concentrations of conjugated dienes, malonic dialdehyde (MDA) [2], and Schiff bases in the liver [15] and MDA content in the hemolysate of erythrocytes [3]. We measured the content of α-tocopherol [10] and vitamin A in the liver [9], amount

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of reduced glutathione [14], and activities of glutathione reductase [13] and glutathione peroxidase in the blood and liver [4,8]. The fractional composition of liver neutral lipids was estimated by thin-layer chromatography after the isolation of lipid extracts [5].

The results were analyzed by Statist software.

RESULTS

Changes in blood lipids in rats feeding the atherogenic diet corresponded to type IIa combined HLP. It was manifested in hypercholesterolemia, increased content of LDL CH, hyper-β-lipoproteinemia, and hypo-α-lipoproteinemia. The content of triglycerides was normal or changed insignificantly (Table 1).

Total lipid content in the liver increased by 13% ($p<0.01$). The development of lipidosis was associated with accumulation of triglycerides, the main fraction of neutral lipids. The imbalance between the intensity of LPO and activity of AOS in the liver and blood indicates that the development of HLP is accompanied by intensification of lipid peroxidation. The content of LPO products considerably increases at various stages of this process (Table 2). Changes in the ratio of oxidation products indicated that intensification of LPO in the liver was more pronounced than in the blood (Table 2). The content of α-tocopherol, the major antioxidant vitamin and functional activity of the glutathione peroxidase+reduced glutathione system inhibiting the formation of lipoperoxides decreased, and activity of glutathione reductase catalyzing the reduction of oxidized glutathione decreased. These changes suggest that functional reserves of AOS in the liver also decreased. Thus, we observed inhibition of the second and third components of AOS and suppression of bioregeneration of oxidized glutathione [7].

Changes in the content of oxidation products indicated that the intensity of LPO in the blood was lower than in the liver, which determined normal functioning of the second components of AOS (Table 2). Consumption of α-tocopherol in the blood was more intensive than in the liver. By contrast, vitamin A content in the blood increased due to mobilization of antioxidant reserves in response to intensive LPO.

Glutathione reductase activity and reduced glutathione content in the blood decreased, while glutathione peroxidase activity remained unchanged. The response of the blood AOS was manifested in activation of its second components (neutralization of lipid radicals with α-tocopherol).

Maksar normalized lipid parameters of the blood and liver in rats with HLP. This preparation most significantly affected the contents of total CH and LDL CH. Their amount decreased by 33 and 41%, respectively ($p<0.01$). The concentrations of blood triglycerides and total lipids tended to decrease. The coefficient of atherogenicity decreased. Maksar produced a lipid-correcting effect on rats with liver lipidosis. The contents of total lipids and triglycerides decreased by 13 ($p<0.01$) and 12.5% ($p<0.05$), respectively.

The intensity of LPO in the liver and blood decreased in animals receiving Maksar. It was manifested in a decrease in the contents of primary, intermediate, and final LPO products (Table 2). Glutathione reductase activity in the liver and blood increased by 41 and 37%, respectively ($p<0.01$). Glutathione

TABLE 1. Lipids of the Blood and Liver in Rats with HLP ($M\pm m$, $n=7$)

Parameters		Control	HLP	HLP+Maksar
Blood	Total CH, mmol/liter	1.48±0.07	2.68±0.06*	1.78±0.02^{+}
	Triglycerides, mmol/liter	0.39±0.03	0.47±0.03	0.44±0.01
	HDL CH, mmol/liter	0.31±0.07	0.23±0.02	0.25±0.02
	LDL CH, mmol/liter	0.99±0.11	2.24±0.08*	1.32±0.02^{+}
	VLDL CH, mmol/liter	0.17±0.01	0.21±0.01	0.19±0.01
	Total lipids, mmol/liter	2.04±0.22	2.48±0.10	2.27±0.04
	Coefficient of atherogenicity	3.77	10.65	6.12
Liver	Total lipids, mg/g tissue	3.58±0.08	40.5±0.8*	3.52±0.09^{+}
Fractions of neutral lipids, %				
	CH	16.80±0.56	13.84±0.82**	12.57±0.33
	free fatty acids	16.07±0.44	16.52±0.58	16.62±0.37
	triglycerides	22.28±0.51	26.04±0.82*	22.76±0.91^{++}
	esterified fatty acids	14.26±0.95	15.20±0.58	14.49±0.09
	CH esters	16.80±0.56	15.35±0.65	16.38±0.36

Note. Here and in Table 2: *$p<0.01$ and **$p<0.05$ compared to the control; $^{+}p<0.01$ and $^{++}p<0.05$ compared to HLP.

TABLE 2. LPO—AOS in the Liver and Blood of Animals with Alimentary HLP (% of Control)

Parameter	Liver		Blood	
	HLP	HLP+Maksar	HLP	HLP+Maksar
Antioxidant activity	—	—	77.5	97.4^{++}
Reduced glutathione	61.8*	75.7^{+}	72.2*	40.6^{+}
Glutathione reductase	68.3*	96.3^{+}	48.1*	63.1^{+}
Glutathione peroxidase	82.4	86.8	105.5	71.5^{+}
Conjugated dienes	251.7*	212.4^{+}	188.8*	128.5^{+}
MDA	225*	151.2^{+}	174.7*	129.8^{+}
Schiff bases	156.5*	137.2^{+}	164.4*	126.7^{+}
α-Tocopherol	75.6*	83.4^{+}	64.3*	74.4^{++}
Vitamin A	90	96	121.9*	95.2^{+}

reductase activity in the liver approached the control level. Maksar significantly increased the amount of reduced glutathione and α-tocopherol in the liver. Vitamin concentration in the blood also underwent considerable changes. The content of α-tocopherol increased, while the concentration of vitamin A returned to the control. Maksar significantly decreased glutathione peroxidase activity in the blood and the content of reduced glutathione in animals with HLP. This is related to the involvement of flavonoids entering the composition of Maksar in the inactivation of free radicals. Moreover, the antiradical effect of external antioxidants partially substitutes for functional activity of the glutathione peroxidase+reduced glutathione system.

Our experiments show that Maksar containing natural antioxidants suppresses LPO in the liver and blood, improves AOS, and corrects changes in blood lipid metabolism and liver lipidosis in animals with type IIa alimentary HLP.

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