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ACID AND NEUTRAL LIPASE ACTIVITY IN LYMPHOCYTES OF PATIENTS WITH INCREASED SERUM CHOLESTEROL AND TRIGLYCERIDE LEVEL

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Acid lipase activity (ALA) and neutral lipase activity (NLA) in lymphocytes of patients with primary hyperlipidemia (hypercholesterolemia or/and hypertriglyceridemia) were compared with that of an age-matched control group (blood donors). The specificity of lipase was confirmed by the use of cardiolipin the well known activator of acidic lipase. β-D-glucuronidase activity was used as a marker of the lysosomal release reaction. ALA (by 33%) and β-D-glucuronidase (by 55%) activity, but not NLA in lymphocytes of the group of hyperlipidemic patients, was significantly lower when compared to the control group. A negative correlation between the serum cholesterol level and ALA, NLA and β-D-glucuronidase release from lymphocytes of hyperlipidemic subjects was observed. The serum HDL cholesterol level was positively correlated with ALA within this group. These results suggest that the high cholesterol level in serum can unspecifically supress ALA and (to the smaller degree) NLA activity in lymphocytes of hyperlipidemic subjects. The decrease of lipase activity may promote deposition of lipids in cells and the development of atherosclerosis. The parallel decrease of β-D-glucuronidase activity in lymphocytes of hypercholesterolemic patients suggests the impairment of immune system in hypercholesterolemia.

Key words: *Acid lipase, β-D-glucuronidase, lymphocytes, hyperlipidemia*

INTRODUCTION

The activity attributed to an enzyme known as lysosomal acid lipase or acid cholesteryl ester hydrolase or acid cholesteryl esterase, (EC 3.1.1.13), plays an important role in lipid metabolism which controls cellular cholesterol turnover (1, 2). A variety of substrates, including cholesteryl esters, triglycerides, methylumbelliferyl and nitrophenyl fatty acyl esters, serve as substrates for its activity. Subcellular fractions have been shown to contain at least three types of intracellular isoforms of this enzyme; a) cytoplasmic with the maximal activity at neutral pH (NLA) (3—8); b) a microsomal

with the highest activity at more acidic pH (9, 10); c) a lysosomal acid lipase (ALA) (7, 8, 11, 12). In the gradually developing form of atherosclerosis, even a slight ALA decrease may contribute to the accumulation of cholesteryl esters in arterial wall (13).

The aim of this study was to answer the question if one of the most important risk factors, such as hyperlipidemia, can influence cellular acid lipase activity in cells which do not participate in lipid catabolism. We determined ALA in lymphocytes of the subjects with primary hyperlipidemia and of the healthy volunteers. Simultaneously neutral lipase activity (NLA) and β-D-glucuronidase activity were measured to ensure the specificity of ALA changes.

MATERIAL AND METHODS

The blood for cell isolation was obtained after 12 hours fasting from 30 normolipidemic, age-matched healthy volunteers, and from 14 patients (45 ± 7 years old men) with primary hyperlipidemia (hypercholesterolemia and/or hypertriglyceridemia) with no abnormalities in tests of renal and hepatic function. Lymphocytes were isolated according to Boyum (14) from heparinized blood and used for the experiment not later than 30 min after blood sampling. The lymphocytes were washed several times with saline and stored at $-80°C$ before use. Lymphocytes and monocytes have the same AL specific activity (15), therefore the contaminating mononuclear cells were not further separated. Lymphocytes were sonificated 3 times for 30 sec at 0°C and centrifuged for 30 min at 4000 g. Total protein in supernatant was determined according to Lowry (16). The protein concentration was adjusted to the same value 1 mg/ml in all the samples studied. Activity of AL and NL was measured fluorometrically at pH 4.3 for acid and at pH 6.9 for neutral lipase with 4-methylumbelliferyl palmitate as substrate (17). Each incubation sample contained 25 ml of 0.4 M acetate buffer, pH 4.3 or pH 6.9 with 0.1% Triton X-100 with or without 1.25% cardiolipin, 50 ml of 10 mM 4-methylumbelliferyl palmitate (Sigma) solubilised in 1.45% Triton X-100 (v/v) and 25 ml of lymphocyte supernatants (corresponding to 25 mg of protein). After 20 min of incubation at 37°C the reaction was stopped by adding 300 ml 5% perchloric acid. The fluorescence of the 4-methylumbelliferone liberated during incubation was developed by the addition of 1.5 ml 0.5 M sodium bicarbonate-sodium carbonate buffer pH 9.5 and read on a spectrofluorimeter (excitation, 335 nm; emission, 445 nm) within two minutes. Enzyme activity is presented in nmol of hydrolized substrate per minute per mg of protein. β-D-glucuronidase activity was measured fluorometrically with 4-methylumbelliferyl -β-D-glucuronide (Sigma) as substrate at pH 4.5 (18). Triglycerides and total cholesterol were measured by means of enzymatic methods (Boehringer Mannheim kits). HDL-cholesterol was determined enzymatically (Boehringer Mannheim kit) in supernate after precipitation of VLDL and LDL fractions with heparin and MnCl2 (19).

Statistical evaluation of the data

Data were expressed as mean $\pm$ SD. Significance of the difference between means was evaluated by Student's t-test.

RESULTS

The two step analysis of obtained results was performed:

a) the first step included the results of all hyperlipidemic patients
b) in the second one, 5 patients with the highest cholesterol level in serum were selected from the above group.

We observed that ALA in lymphocytes of patients with primary hyperlipidemia was significantly lower (5.54 ± 1.72 nmol/min/mg protein) than in a normolipidemic group (8.03 ± 1.6 nmol /min/mg protein). In the case of 5 selected patients with the highest cholesterol level in serum, the ALA and NLA were much more decreased than in the whole hyperlipidemic group (*Table 1*). The addition of cardiolipin to the incubation mixture increased ALA

Table 1. Serum lipids parameters and lymphocytes ALA and NLA (with and without cardiolipin), β-D-glucuronidase activity in normo- and hyperlipidemic subjects.

Subjects		Serum			Lymphocytes				
		CH	TG	HDL-CH	ALA	ALA*	NLA	NLA*	β-gluc
		mmol/l			nmol/min/mg protein				
normolipemic	x	5.50	1.47	1.40	8.03	17.26	2.37	2.25	6.86
n = 30	sd	1.10	0.75	0.27	1.60	3.12	0.11	0.40	0.26
hyperlipemic	x	9.07	6.97	1.00	5.54	11.02	2.01	1.36	3.08
n = 14	sd	1.42	6.39	0.27	1.72	2.45	0.69	0.56	0.56
		***	***	***	***	***		***	***
hyperlipemic	x	10.50	3.93	0.97	3.61	8.36	1.19	0.78	3.25
n = 5*	sd	1.82	3.19	0.07	0.77	1.42	0.29	0.19	0.78
		***	***	***	***	***	***	***	***

Normo- vs hyperlipemic groups: *** – $p < 0.001$
Abbreviations: CH – total cholesterol; TG – triglycerides;
HDL-CH – HDL-cholesterol; ALA – acid lipase activity;
NLA – neutral lipase activity; * – cardiolipin in incubation mixture;
β-gluc. – β-D-glucuronidase activity;
n = 5* – hyperlipidemic subjects with the highest total cholesterol level in serum.

about 2 times in a similar way in normo- and in hyperlipidemic group. No influence of cardiolipin on NLA within all investigated groups was observed (17). β-D-glucuronidase activity was also significantly decreased in hyperlipidemic patients (*Table 1*). Contrary to ALA and NLA, there was no more decrease of β-D-glucuronidase activity in 5 patients with the highest cholesterol level in serum (*Table 1*). The values presented in *Table 2* indicate the negative correlation between the total cholesterol level in serum and ALA, NLA and β-D-glucuronidase activity in lymphocytes of patients with primary hyperlipidemia, and positive correlation of HDL cholesterol fraction in serum with ALA of lymphocytes.

Table 2. Correlation coefficients between serum lipid parameters and lymphocyte enzyme activities in hyperlipidemic patients.

Lymphocytes	Serum		
	CH	Tg	HDL-CH
ALA	−0.546*	0.042	0.515*
NLA	−0.526*	0.216	0.394
β-D-gluc.	−0.452*	−0.141	0.149

* – $p<0.05$

DISCUSSION

Contradictory data concerning the acid lipase (ALA) in lipid-loaded cells have been described. Yatsu et al. (20) and Coray et al. (21) demonstrated depressed activity of ALA in mononuclear cells in individuals with symptomatic atherosclerosis, while Brecher et al. (22) and Haley et al. (23) observed increase of this enzyme activity in lipid-loaded aortic smooth muscle cells. However, this discrepancy may be explained by a different kind of cells used (24). We found that lymphocyte AL activity of patients with primary hyperlipidemia was significantly lower. Our findings are in agreement with those observed in patients with atherosclerosis (22, 23) and in experimental diabetes with concomitant increased cholesterol and lipoprotein levels in the blood (25). Decreased ALA in lymphocytes in hyperlipidemia could be related to the elevation of intracellular cholesterol concentration rather than to the inborn enzyme deficiency. We observed a negative correlation between the total cholesterol concentration in serum and ALA and NLA in lymphocytes. The ability of cardiolipin to stimulate ALA equivalently (by 2 times) in lymphocytes obtained both from control and from hyperlipidemic subjects excluded the inborn deficiency of ALA. The release of β-D-glucuronidase was also decreased by hypercholesterolemia which suggests that the other function of lymphocytes can be impaired by the increased level of lipids in the blood. Indeed, the disturbances in immunological response in atherosclerosis was demonstrated (26, 27).

To summarize our results, it may be concluded that the high total cholesterol concentration in serum of patients with hyperlipidemia results in non specific supression of acid and neutral lipase activity in lymphocytes, that might be aditionally aggravated by the decrease of the serum HDL fraction (also observed in our study). We assume that the supression of lipase activity in lymphocytes of patients with hypercholesterolemia may lead to the deposition of lipids in other cells than macrophage and myocyte. Such lipids deposition in lymphocytes results in the impairement of their function (s).

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