Effects of Lipoic Acid on Proliferation and Apoptosis of Liver Cells in Rats with Metabolic Stress

E. A. Martinova, A. V. Vasiliev, N. E. Sharanova, and M. M. Gapparov

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Lipoic acid stimulated expression of heat shock proteins 25, 70, and 90 in liver cells of Wistar rats with metabolic stress (5 days of food deprivation followed by complete resumption of nutrition). Lipoic acid in a dose of 25 mg/kg reduced proliferation of hepatic lymphocytes during fasting, while after resumption of feeding it stimulated hepatocyte proliferation due to differentiated regulation of the expression of cyclin D1 and Rb protein in these cell populations.

Key Words: *lipoic acid; liver; stress; apoptosis; proliferation*

R(+)-α-Lipoic acid (1,2-dithiolan-3-pentanic; LA) an antioxidant and enzyme cofactor, is synthesized in mitochondria. It is used in the therapy of hepatitis, diabetes mellitus, neurodegenerative diseases, and cancer. LA inhibits lipogenesis in steatohepatosis [3]. Lipoic acid elevates serum levels of S-adenosine homocystein, cystathionine, and N,N-dimethylglycine, reduces hepatic levels of S-adenosine methionine and NADPH cytochrome P450 reductase activity [9], and improves glucose utilization by cells due to stimulation of insulin-dependent Akt/PKB signal pathway [8]. In addition, it improves mitochondrial membrane potential and cell survival due to stimulation of the transcription of antioxidant genes [6].

We studied the effects of LA on stress proteins, proliferation and apoptosis of rat liver cells in metabolic stress induced by fasting and sharp restoration of nutrition.

MATERIALS AND METHODS

The study was carried out on 10-week-old female Wistar in accordance with international regulations on handling of laboratory animals. Food deprivation for 5 days with free access to water, followed by complete restoration of nutrition for the next 5 days, served as the stress model. The animals were randomly divided into groups (5 animals per group). Controls (group 1) received vivarium ration, other groups were subjected to food deprivation (group 8 was intact control 2). Groups 3 and 6 received LA orally in a daily dose of 2.5 mg/kg in 50 μg vegetable oil, groups 4 and 7 rats received LA in a dose of 25 mg/kg. Groups 2 and 5 received 50 μg vegetable oil orally. After 5 days, animals of groups 1-4 were sacrificed by guillotine. Animals of groups 5-8 received vivarium ration for the next 5 days. Manipulations with organs and cells were carried out as described previously [1] (Fig. 1). Experiments were repeated 3 times, 5 animals per study point, the results were presented as $M \pm m$. The groups were compared by pairs using Student's test, the data were processed by the ANOVA software. Nonparametric analysis of several groups was carried out by multiple comparison by the Newman–Keuls method. The differences between the groups were considered significant at $p<0.05$.

RESULTS

Lipoic acid in a dose of 25 mg/kg significantly prevented body weight drop during complete food deprivation and promoted body weight gain after fasting was discontinued (Table 1). A trend to liver weight gain was observed in rats receiving LA in a dose of 25 mg/kg in comparison with fasting animals.

Institute of Nutrition, the Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** e.a.martinova@gmail.com. E. A. Martinova

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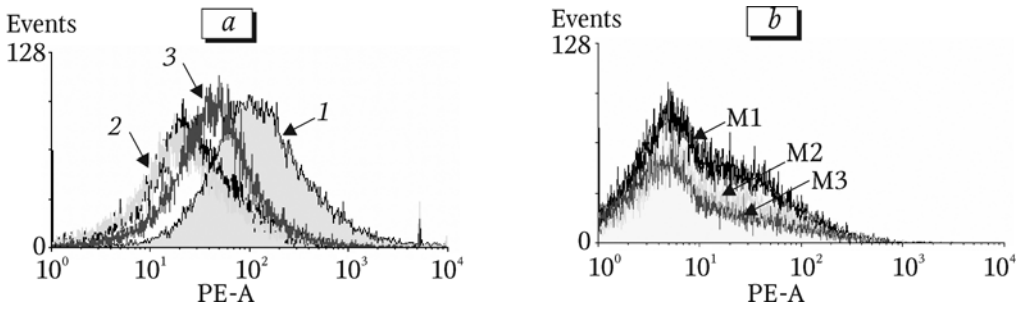

Fig. 1. Analysis of intracellular protein expression by flow cytometry. *a*) changed expression of Hsp: lesser peak areas and fluorescence intensities in histograms *2* and *3* compared to the control (*1*); *b*) changed expression of Rb protein in liver cells under the effects of LA in different doses (markers M2 and M3) *vs.* control (marker M1).

Heat shock proteins (Hsp) have several functions during stress, react with second messengers of cellular signal pathways, and modify physical properties of proteins. Lipoic acid stimulates the expression of Hsp72 and Hsp25 in skeletal muscles and restores cellular response to insulin in rats receiving fat-rich diets [4]. Our data indicate that LA treatment during fasting significantly stimulates the expression of Hsp25 and Hsp90 and induces a trend to elevation of Hsp70 expression. The expression of these proteins increased significantly after fasting was discontinued, which attested to improvement of the adaptation potential (Table 2).

Analysis of liver cells on a flow cytometer showed changes in their population composition during food deprivation and LA treatment (Fig. 2, *a*). Lipoic acid in a dose of 25 mg/kg caused marked enlargement of hepatocyte pool and increased proliferative activity of these cells.

TABLE 1. Effects of Lipoic Acid on Body Weight and Liver Weight of Experimental Rats (*M*±*m*)

Parameter		Control	Food deprivation, 5 days			Food deprivation, 5 days+100% ration, 5 days		
			food deprivation	2.5 mg/kg LA	25 mg/kg LA	food deprivation	2.5 mg/kg LA	25 mg/kg LA
Body weight	before experiment	157±7	151±9	151±10	157±8	151±10	156±6	157±6
	after experiment	232±22	131±15	137±16	156±16*	162±18	174±18	195±19*
Body weight changes, %		+47±8	-10.8±6.0	-9.5±3.0	-0.5±0.4**	+10.2±5.0	+12.6±2.0	+25±4.0*
Liver weight after experiment		10.0±1.2	4.8±0.5	4.8±0.5	5.5±0.7	7.9±1.5	8.0±0.8	9.0±1.4

Note. Here and in Table 2: *$p \le 0.05$, **$p \le 0.001$ compared to the control.

TABLE 2. Effects of Lipoic Acid on Hsp Expression (%) in the Rat Liver (*M*±*m*)

Parameter	Control	Food deprivation, 5 days			Food deprivation (5 days)+100% ration (5 days)		
		food deprivation	2.5 mg/kg LA	25 mg/kg LA	food deprivation	2.5 mg/kg LA	25 mg/kg LA
Hsp25	11±2	13±3	16±4	19±3*	23±6	25±3	28±5*
Hsp70	16±4	22±5	25±3	28±4	33±3	38±5	41±3*
Hsp90	1.3±0.4	2.9±0.2	5.2±0.5	9.3±1.3**	4.3±0.4	7.7±0.7	8.1±0.9**

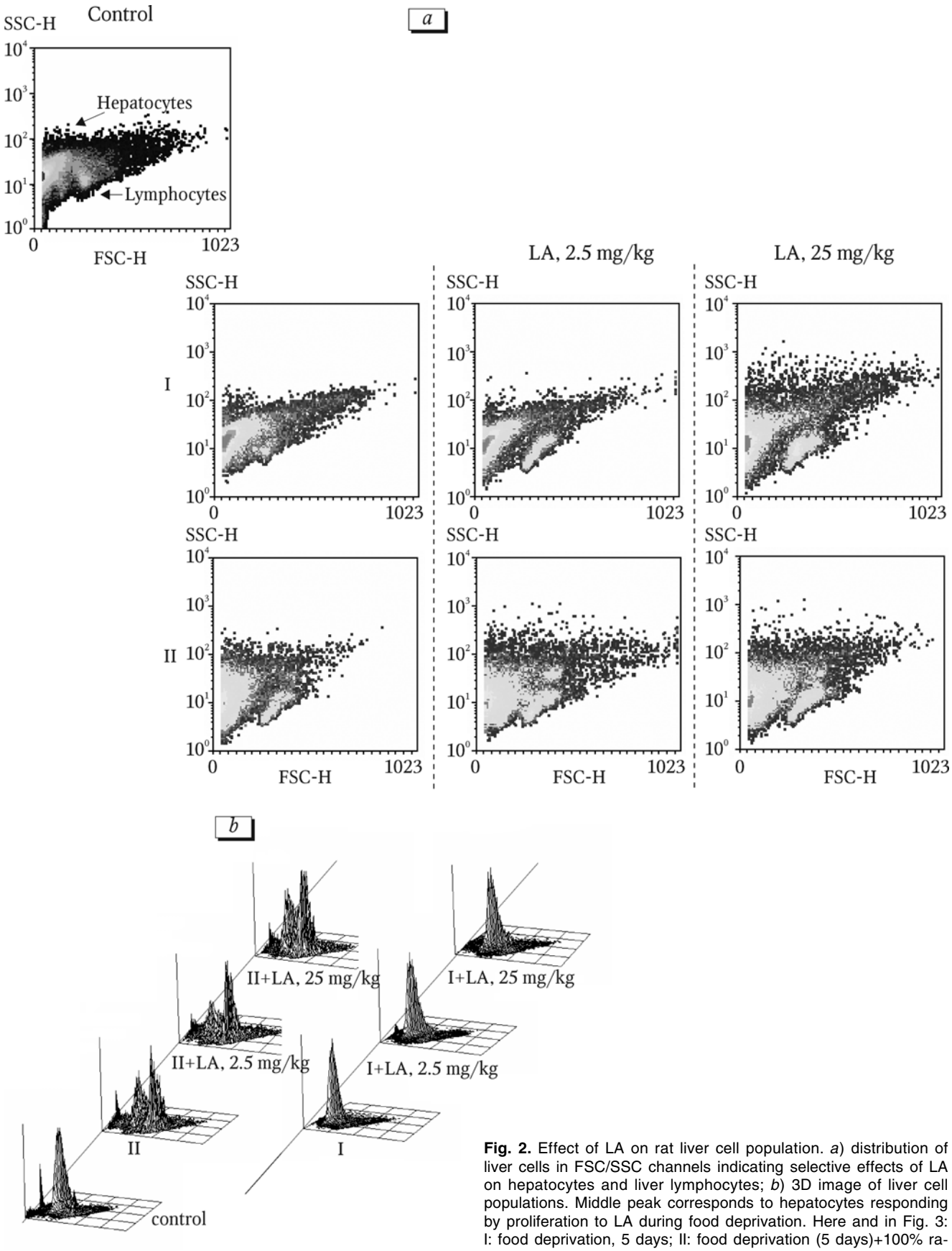

Fig. 2. Effect of LA on rat liver cell population. *a*) distribution of liver cells in FSC/SSC channels indicating selective effects of LA on hepatocytes and liver lymphocytes; *b*) 3D image of liver cell populations. Middle peak corresponds to hepatocytes responding by proliferation to LA during food deprivation. Here and in Fig. 3: I: food deprivation, 5 days; II: food deprivation (5 days)+100% ration (5 days).

TABLE 3. Effects of Lipoic Acid on Liver Cell Proliferation in Rats with Metabolic Stress (*M*±*m*)

Parameter	Control	Food deprivation, 5 days			Food deprivation (5 days)+100% ration (5 days)		
		food deprivation	2.5 mg/kg LA	25 mg/kg LA	food deprivation	2.5 mg/kg LA	25 mg/kg LA
Lymphocytes							
Lymphocytes, %	10.4±1.3	52±2.0	10.6±4.2	18.1±8.4	20.5±0.6	21.2±5.2	15.3±4.1
G1 phase, %	55.4±4.6	45.0±5.4	67.0±9.8	77.1±9.9	70.4±6.6	73.9±8.0	69.5±5.1
S phase, %	17.3±2.2	22.8±2.1	12.3±2.2	9.0±4.1	14.3±2.0	12.7±2.2	15.4±2.6
G2/M phase, %	26.2±3.5	31.8±6.0	19.5±6.0	13.8±8.0	15.1±4.4	13.2±3.8	15.2±3.7
Cyclin D1, %	15.7±2.6	15.3±1.0	11.5±2.7	10.2±3.9	14.2±3.1	13.6±2.0	14.5±2.7
p-Rb protein, %	4.1±0.5	5.4±0.5	4.0±0.6	4.0±0.8	3.9±0.9	3.8±0.9	4.0±0.7
Mitoses, %	3.8±0.4	3.3±0.4	3.1±0.3	2.6±0.5	4.4±0.6	3.9±0.8	4.8±0.5
Apoptosis, %	2.4±0.9	5.7±1.5	3.3±0.8	4.2±1.0	3.9±1.1	3.6±0.9	4.3±1.2
DNA, mean	87±4	111±6	76±7	82±5	91±6	111±8	121±8
Hepatocytes							
G1 phase, %	77.3±2.9	73.2±5.3	85.4±2.5	86.5±4.7	85.7±2.6	78.5±5.3	81.5±2.4
S phase, %	13.8±3.9	16.3±5.8	8.3±1.4	6.7±3.4	14.3±1.9	13.7±2.6	15.6±2.4
G2/M phase, %	7.9±2.6	10.2±4.1	6.0±1.3	6.9±3.2	4.7±1.2	4.5±1.7	6.9±0.8
Cyclin D1, %	14.0±2.4	20.0±4.5	10.0±1.7	8.0±2.1	18.0±5.1	16.0±4.0	17.0±3.7
p-Rb protein, %	4.5±0.7	4.7±0.6	4.1±0.5	4.1±0.5	4.7±0.6	6.9±1.3	7.2±1.1
Mitoses, %	5.6±0.9	4.9±0.7	3.8±0.4	3.8±0.5	4.2±0.4	6.9±0.9	7.2±0.9
Apoptosis, %	4.6±1.3	7.9±3.2	4.4±2.5	5.0±1.9	5.1±1.7	4.8±1.8	4.4±1.9
DNA, mean	81±4	96±4	75±6	80±4	102±7	124±9	129±5

Note. Mean: fluorescence intensity.

Injection of LA during fasting reduces the percentage of dividing lymphocytes in the liver (control: 54%; LA, 2.5 mg/kg: 32%; LA, 25 mg/kg: 22%), which reduces the risk of autoimmune diseases (Fig. 2, *b*). This is true for S phase and G2/M phase cells. The bulk of proliferating cells are B cells. Importantly that the experiment was carried out on young rats aged 8-10 weeks, which, in contrast to adult animals, are characterized by high proliferation of the gastrointestinal tract cells during food deprivation [7]. Lipoic acid did not stimulate liver lymphocytes during nutrition resumption after fasting (Fig. 3).

Treatment with lipoic acid during food deprivation leads to reduction of hepatocyte percentage paralleled by reduction of expression of cyclin D1 (Table 3) regulating cell transition from late G1 to S phase and arrests the cell cycle in G1 phase. These events are paralleled by inhibition of cyclin E/cdk2 complex and hypophosphorylation of Rb protein (retinoblastoma gene protein). Rb protein is a regulatory protein in the G1/S phase transition requiring the presence of cyclin D1, p27 inhibitor, and stimulation of B/Akt protein kinase [2]. Hepatocyte proliferation is also regulated by kinases Pl3K, MLCK, ERK2, p70S6K1, cdk2, by NF-kB transcription factor, *etc.* [5]. In contrast to lymphocytes, in hepatocytes LA stimulates the expression of Rb protein during nutrition restoration after food deprivation (Table 3), which leads to stimulation of hepatocyte proliferation in comparison with the control (Fig. 3).

Hence, the effects of LA on hepatocytes and liver lymphocytes are different because of different regulation of cyclin D1 and Rb protein expression. Stimulation of hepatocyte proliferation after liver weight loss during food deprivation is an adaptive mechanism supporting normal function of the organ.

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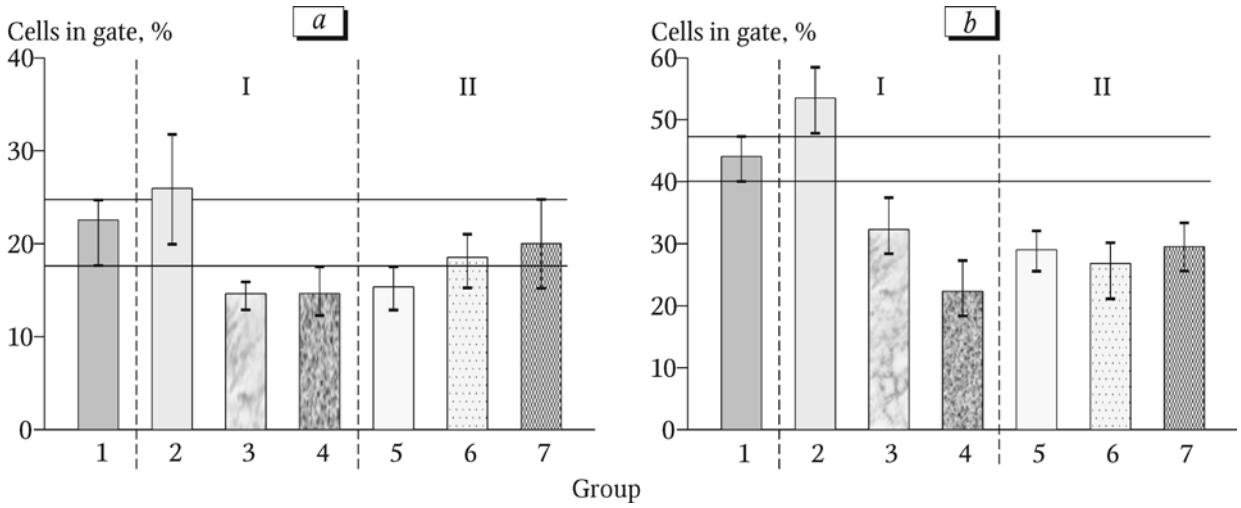

Fig. 3. Lipoic acid dose-dependent reduction of lymphocyte proliferation in the liver during food deprivation. *a*) hepatocytes (% of dividing cells); *b*) lymphocytes (% of dividing cells).

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