Effects of Silymarin (Hepatoprotector) and Succinic Acid (Bioenergy Regulator) on Metabolic Disorders in Experimental Diabetes Mellitus

A. I. Vengerovskii, V. A. Khazanov*, K. A. Eskina, and K. Yu. Vasilyev*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 7, pp. 58-61, July, 2007
Original article submitted September 12, 2006

Silymarin (70 mg/kg) and succinic acid (50 mg/kg) reduce blood glucose and cholesterol concentrations, inhibit LPO, and correct oxidative phosphorylation disturbances in liver mitochondria in experimental diabetes mellitus induced by injection of streptozotocin (65 mg/kg) in rats.

Key Words: *silymarin; succinic acid; streptozotocin; liver mitochondria*

Pathologies of the liver develop in 34-75% patients with diabetes mellitus [2]. Citric acid cycle disturbances, partial uncoupling of oxidation and phosphorylation, accumulation of highly toxic lipoperoxide compounds destabilizing membranes are observed in hepatocyte mitochondria (MC) of diabetics [9]. Hepatoprotectors (silymarin containing holy thistle (*Silybum*) flavolignanes) and bioenergetic regulators (succinic acid, SA) improving liver functions are characterized by potential therapeutic activity in diabetes mellitus [4,6]. Silymarin characterized by a direct antioxidant effect prevents the destruction of acinuses and pancreatic islets in experimental pancreatitis, normalizes metabolic processes in the liver, and reduces blood glucose level [5].

We studied the possibility of correcting metabolic disorders in experimental streptozotocin-induced diabetes mellitus with silymarin (hepatoprotector) and SA (energy metabolism regulator).

Department of Pharmacology, Siberian State Medical University, Tomsk; *Laboratory of Molecular Pharmacology, Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Sciences, Tomsk. ***Address for correspondence:*** edu@ssmu.ru. A. I. Vengerovsky

MATERIALS AND METHODS

Experiments were carried out in winter-spring on 60 outbred male rats (200-220 g) from Clinic of Laboratory Animals, Institute of Pharmacology. The animals were kept under standard vivarium conditions at natural illumination with free access to water and fodder. The studies were carried out in accordance with recommendations on experimental trials of new drugs [3].

The rats received a single intraperitoneal injection of streptozotocin (ISN) in a dose of 65 mg/kg [8]. Starting from day 2 after streptozotocin injection, the rats received silymarin (Madaus; 70 mg/kg) or SA (50 mg/kg) in the form of suspension in 1% starch gel intragastrically for 14 days. These drug doses are therapeutically effective [5,6]. Controls received starch gel. The rats were decapitated under light ether narcosis 12 h after the last drug dose.

Serum and liver homogenate were used in the study. Serum glucose was measured by the glucose oxidase method, total protein by the biuret method, and total cholesterol by enzymatic colorimetric method. The function of liver MC was evaluated by the rate of oxygen consumption measured by the polarographic method on a PA-2 polarograph with

a Clarke electrode made in laboratory [7,10]. The rates of oxygen consumption before (V_{4P}), during (V_3), and after (V_{4O}) cycle of exogenous ADP (10^{-4} M) during oxidation of phosphorylation of endogenous substrates, flavine-dependent substrate (succinate, 10^{-3} M), and NAD-dependent substrates (malate and glutamate, 3×10^{-3} M each) were calculated. Malonate (SDH inhibitor, 2×10^{-3} M) and aminooxyacetate (aminotransferase inhibitor, 5×10^{-4} M) were used. The MC energy status was evaluated by the coefficients of respiration stimulation ($RS=V_3/V_{4P}$), respiration control ($RC=V_3/V_{4O}$), and oxidative phosphorylation coupling (ADP/O). Activity of LPO was evaluated by the rates of spontaneous and ascorbate-dependent MDA formation and content of conjugated dienes and Schiff's bases.

The results were processed using Mann—Whitney test.

RESULTS

Toxic effect of streptozotocin on pancreatic β-cells is associated with insulin deficiency [8]. On day 2 after streptozotocin injection, the rats developed metabolic disorders characteristic of diabetes mellitus. Blood glucose concentration increased 3-fold, cholesterol concentration increased 1.7 times, and protein level decreased by 1.7 times in comparison with the levels in control animals (Fig. 1).

Respiration rates increased in liver MC during oxidation of malate and glutamate substrates in rats injected with streptozotocin in comparison with normal level in all metabolic states. The RS and RC coefficients decreased, ADP/O coefficient during oxidation of NAD-dependent substrates increased in comparison with the normal, which indicated activation of succinate-dependent energy production. Inhibitory analysis of NAD-dependent respiration using malonate confirmed the hyperactive status of liver MC (the rates of selective NAD-dependent respiration and the contribution of endogenous succinate to energy production increased). Respiration rates increased after addition of aminooxyacetate to MC incubation medium, which was paralleled by a decrease in the RS coefficient (Table 1). These data indicate the development of hypercompensated low-energy shift in liver MC during streptozotocin into-

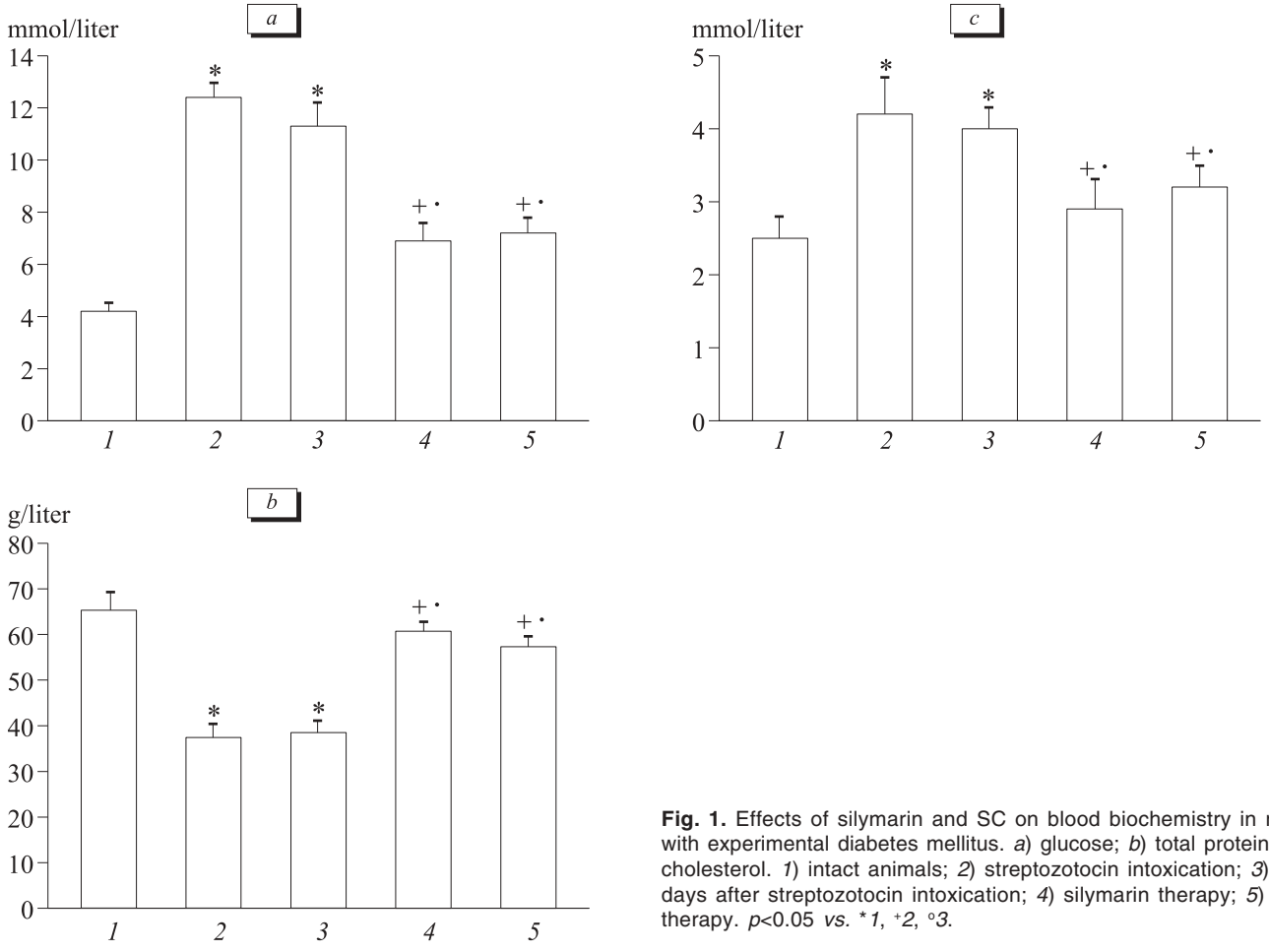

Fig. 1. Effects of silymarin and SC on blood biochemistry in rats with experimental diabetes mellitus. *a*) glucose; *b*) total protein; *c*) cholesterol. *1*) intact animals; *2*) streptozotocin intoxication; *3*) 14 days after streptozotocin intoxication; *4*) silymarin therapy; *5*) SA therapy. $p<0.05$ *vs.* **1*, +*2*, °*3*.

TABLE 1. Effects of Silymarin and SA on Oxidative Phosphorylation and LPO in the Liver in Experimental Streptozotocin-Induced Diabetes Mellitus ($M \pm m$; n=10)

Parameter	Control animals	Streptozotocin	14 days after streptozotocin injection	Therapy	
				silymarin	SA
Succinate oxidation					
V_{4P}	36.1±1.9	65.6±5.4*	62.3±1.3*	42.4±1.9^{+o}	38.5±1.7^{+o}
V_3	81.9±2.8	114.0±4.9*	115.6±5.8*	74.2±3.1^{+o}	82.9±3.3^{+o}
V_{4O}	38.7±1.6	64.9±4.3*	58.6±2.3*	40.9±1.2^{+o}	43.6±1.3^{+o}
ADP/O	2.56±0.14	1.63±0.11*	1.59±0.11*	2.36±0.04^{+o}	1.83±0.10*
Oxidation of NAD-dependent substrates (malate+glutamate)					
V_{4P}	22.1±1.4	32.4±1.9*	30.1±1.3*	23.5±1.9^{+o}	20.4±1.5^{+o}
V_3	56.5±1.4	78.5±2.5*	75.3±2.7*	57.7±1.4^{+o}	68.8±2.9^{*+o}
V_{4O}	17.5±1.9	35.1±1.7*	32.9±1.8*	21.8±1.1^{+o}	24.0±1.7^{*+o}
ADP/O	2.16±0.10	2.57±0.10*	2.53±0.05*	1.99±0.08^{+o}	2.69±0.07*
Oxidation of NAD-dependent substrates in the presence of malonate					
V_{4P}	16.3±1.7	34.2±1.6*	39.6±1.0*	20.2±1.1^{+o}	17.6±1.6^{+o}
V_3	42.9±2.2	77.7±4.9*	76.7±4.1*	45.4±2.2^{+o}	44.9±3.1^{+o}
V_{4O}	17.6±1.6	33.1±1.3*	31.0±1.7*	23.7±2,8^{+o}	17.4±1.6^{+o}
ADP/O	1.90±0.08	2.31±0.12*	2.69±0.07*	1.68±0.15^{+o}	2.55±0.11*
Oxidation of NAD-dependent substrates in the presence of aminohydroxyacetate					
V_{4P}	18.3±1.8	32.7±2.4*	31.1±1.5*	17.0±1.4^{+o}	23.6±1.6^{+o}
V_3	56.7±2.1	87.1±6.3*	85.3±4.1*	55.6±1.9^{+o}	51.8±3.9^{+o}
V_{4O}	20.8±1.2	32.5±2.1*	29.5±1.9*	22.5±1.7^{+o}	20.8±1.3^{+o}
ADP/O	2.03±0.05	2.38±0.10*	2.63±0.10*	2.08±0.07^{+o}	2.34±0.08*
LPO parameters					
MDA, nmol/mg protein/min:					
ascorbate-dependent	1.0±0.1	2.3±0.1*	2.6±0.1*	1.4±0.1^{+o}	1.6±0.2^{*+o}
spontaneous	1.2±0.1	2.7±0.1*	2.9±0.2*	1.6±0.3^{+o}	2.1±0.12^{*+o}
Conjugated dienes, U/mg lipids	2.6±0.2	6.3±0.3*	7.1±0.2*	2.8±0.2^{+o}	4.1±0.2^{+o}
Schiff's bases, U/mg lipids	4.1±0.2	6.2±0.2*	6.9±0.1*	4.4±0.2^{+o}	4.8±0.1^{+o}

Note. p<0.05 compared to: *control animals, $^{+}$streptozotocin, ^{o}14 days after streptozotocin injection. Respiration rates (V_{4P}, V_3, V_{4O}) are calculated as nanogram-atom O_2/min/mg mitochondrial protein.

xication caused by increased permeability of MC membranes for H^+ and uncoupling of oxidative phosphorylation [6]. The intensity of LPO in liver homogenate increased: the production of MDA, levels of conjugated dienes and Schiff's bases increased by 1.5-2.4 times (Table 1).

On day 14 after single injection of streptozotocin, blood levels of glucose, cholesterol, and protein, parameters of oxidative phosphorylation in MC, and LPO in liver homogenate remained practically the same as during the acute period of damage to pancreatic islets (Table 1, Fig. 1).

Under the action of silymarin and SA treatment, blood glucose and cholesterol concentrations in rats injected with streptozotocin decreased by 1.3-1.8 times and protein level increased by 1.5-1.6 times in comparison with untreated animals. Despite significant regression of these biochemical disorders, blood values characterizing metabolic effects of insulin did not return to normal (Fig. 1).

Utilization of exogenous succinate and NAD-dependent substrates in rats treated by silymarin during 14 days after streptozotocin injection was associated with normalization of liver MC respira-

tion rates at rest, during ADP phosphorylation and at rest, and by normalization of the ADP/O coefficient. Inhibitory analysis of MC respiration using malonate and aminooxyacetate showed that silymarin therapy did not modify the production of endogenous succinate, selective NAD-dependent oxidation processes, and oxidative phosphorylation in comparison with control animals (Table 1).

Therapy with SA also significantly improved liver bioenergetics in rats with streptozotocin intoxication. The MC respiration rates, RS and RC coefficients normalized in experiment with oxidation of succinate added to the incubation medium, while the ADP/O coefficient remained low. These changes indicate recovery of succinate-dependent reactions in the liver energy production system under the effect of SA. The MC respiration rates decreased by 9-37%, RS, RC, and ADP/O coefficients increased 1.3-1.4 times during oxidation of NAD-dependent substrates in comparison with the values recorded in experimental diabetes mellitus, the V_3 and V_{4O} rates even surpassed the normal. The inhibitory analysis showed that production and oxidation of succinate predominated in respiratory activity in comparison with the NAD-dependent processes in the liver MC in rats treated with SA (Table 1).

Silymarin and SA effectively prevented streptozotocin-stimulated accumulation of LPO products in liver homogenate. Silymarin normalized the rate of MDA production and content of conjugated dienes and Schiff's bases. LPO parameters during SA treatment surpassed the normal (Table 1).

Hence, silymarin (hepatoprotector of the free radical scavenger group) and SA (energy metabolism regulator) exhibited therapeutic effects in diabetes mellitus induced by injection of streptozotocin. These drugs reduce hyperglycemia, hypercholesterolemia, and hypoproteinemia, inhibit production of LPO products in liver tissue, and optimize (to a different degree) mitochondrial oxidation. Silymarin flavolignanes (active antioxidants) increase permeability of hepatocyte membrane for glucose, activate RNA polymerase in hepatocyte nuclei and increase RNA transcription and protein synthesis [4]. In addition, silymarin reduces streptozotocin-induced deenergization of liver MC and restores coupling of oxidation and phosphorylation. Succinic acid directly stimulates insulin synthesis by stimulating the metabolic processes in the pancreatic islets [1]. As a result of its insulin-like effect, SA activates SDH in liver MC and activates rapid metabolic cluster providing maximum production of ATP [10].

REFERENCES

1. A. M. Babskii, Yu. S. Stefankoff, and V. M. Korobov, *Ukr. Biokhim. Zh.*, **56**, No. 6, 106-108 (1993).
2. A. Ya. Nikolaev and E. V. Osipov, *Biochemistry of Insulin-Dependent Diabetes Mellitus* [in Russian], Moscow (2000).
3. *Manual of Experimental (Preclinical) Studies of New Drugs* [in Russian], 2nd ed., revised and suppl., Moscow (2005).
4. L. I. Samigullina and D. N. Lazareva, *Eksp. Klin. Farmakol.*, **67**, 77-80 (2004).
5. A. S. Saratikov, A. I. Vengerovskii, M. E. Mozjelin, and I. V. Sukhodolo, *Khim.-Farm. Zh.*, **35**, No. 12, 6-7 (2001).
6. V. A. Khazanov, *Energy Metabolism Regulators* [in Russian], Tomsk (2004), pp. 4-7.
7. V. A. Khazanov and K. Yu. Vasilyev, *Byull. Eksp. Biol. Med.*, Suppl. 1, 54-60 (2005).
8. J. W. Cardinal, D. J. Allan, and D. P. Cameron, *Eur. J. Med. Res.*, **7**, No. 3, 65-75 (1999).
9. S. H. Caldwell, R. H. Swerdlow, E. M. Khan, *et al.*, *J. Hepatol.*, **31**, No. 3, 430-434 (1999).
10. M. N. Kondrashova, *Eur. J. Med. Res.*, **5**, No. 1, 58-68 (2000).