Antioxidant and Endothelium-Stabilizing Effects of Simvaglyzin on Rabbits with Experimental Hypercholesterolemia

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Simvaglyzin, a complex compound of simvastatin and glycyrrhizic acid, administered to rabbits with experimental hypercholesterolemiain in doses equivalent to 66.6 and 40 μg/kg simvastatin exhibited antioxidant capacity (decreased the content of lipid peroxidation products in the blood by 27-41%) and endothelium-normalizing effect (decreased the level of von Willebrand factor and endothelin-1 by 26-58 and 21-29%, respectively, compared to 200 μg/kg simvastatin, $p<0.05$).

Key Words: *simvaglyzin; simvastatin; hypercholesterolemia in rabbits; antioxidant effect; endothelial dysfunction*

Hypercholesterolemia (HCS) plays an important role in the pathogenesis of atherosclerosis and coronary heart disease (CHD) [7]. The morbidity and mortality rate from these diseases remain high in Russia [3]. Treatment with cholesterol-lowering drugs is a priority strategy in the therapy of CHD and HCS. 3-Hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) inhibitors, statins, are most potent in reducing the content of low-density lipoprotein cholesterol and mortality rate from atherosclerosis and CHD [2]. Besides hypocholesterolemic activity, statins have pleiotropic properties [7], exhibit antioxidant capacity [13], and normalize endothelial function [11]. Therefore, the search and development of new drugs with these properties is an urgent problem.

One of the approaches to the development of new pharmaceutical drugs is the formation of complexes from pharmaceutics and natural complexons, including glycyrrhizic acid (GA) [5]. This approach was used to synthesize a new molecular complex of simvastatin (SV) and GA, simvaglyzin (SVG) [1]. Previous studies on rabbits with experimental HCS showed that the hypocholesterolemic effect of SVG in doses equivalent to 40, 66.6, and 100 μg/kg SV is similar to that of 200 μg/kg SV [4]. It should be emphasized that myotoxicity of SVG was lower than that of SV (estimated from blood creatine phosphokinase activity).

Here we studied the *in vivo* antioxidant and endothelium-stabilizing effects of SVG on rabbits with experimental HCS.

MATERIALS AND METHODS

The study was performed on male rabbits (n=25, Gray Giant) weighing 2.5-3.0 kg. The time of experiments was 80 days. The animals were housed in individual cages and had free access to water and

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food. For modeling HCS, the animals fed a diet containing 5% animal fat and 3% cholesterol for 30 days [9,10]. The rabbits were divided into 5 equal groups, which received hypocholesterolemic drugs and fed a standard laboratory diet on days 31-60: no treatment (control, group 1); SV in a dose of 200 μg/kg (group 2); and SVG in doses of 1000, 666, and 400 μg/kg (groups 3, 4, and 5, respectively). The weight ratio of SV in SVG is 0.1 of molar mass. These doses of SVG in groups 3, 4, and 5 corresponded to 100, 66.6, and 40 μg/kg SV, respectively. Hence, SVG doses in these animals were lower than SV dose in group 2 rabbits (by 2, 3, and 5 times, respectively). The test preparations in 1% starch gel were given perorally once a day. On days 61-80, the animals fed standard laboratory diet. On days 30, 40, 50, 60, 70, and 80 of the study, the blood (3 ml) was taken from the ear vein in the morning. The animals were deprived of food, but had free access to water over 12 h before blood sampling.

Total cholesterol level in blood plasma was measured on a Labsystem F-900 biochemical analyzer using Biocon kits. The content of lipid peroxidation (LPO) products in blood plasma was estimated from the concentration of thiobarbituric acid-reactive substances. Fluorometry was performed on a SpectroFluor spectrofluorometer (Bio-Rad). Paraoxonase activity in blood plasma was measured photometrically using Tris-HCl buffer (pH 8.0) [12]. Endothelial function was estimated from plasma levels of NO (total nitrite concentration, R&D Systems for enzyme immunoassay, detection at 540 nm), von Willebrand factor (Technoclone test system for enzyme immunoassay, detection at 450 nm), and endothelin-1 (Biomedica Group test system for enzyme immunoassay, detection at 450 nm). The measurements were performed on a Multiscan EX enzyme immunoassay analyzer.

Statistical treatment of data involved SPSS for Windows software. The results were analyzed by correlation analysis, one-way analysis of variance (ANOVA), and Dunnett test for multiple comparison. The differences were significant at $p<0.05$.

RESULTS

Feeding a cholesterol-rich diet over the first 30 days was followed by the development of severe HCS in rabbits (total blood cholesterol level 2309.0± 223.2 mg/ml). Previous studies showed that SV inhibits oxidative processes and normalizes endothelial function in patients with HCS [11,13]. The antioxidant and endothelium-stabilizing effects of SV and SVG were studied on this experimental model.

Studying the antioxidant effects of the test preparations showed that the content of LPO products in the blood decreased by 23-31% after SV administration for 20 days ($p<0.05$ compared to the control; Fig. 1, *a*). We evaluated the effect of SVG in doses of one-third and one-fifth of SV. On day 10

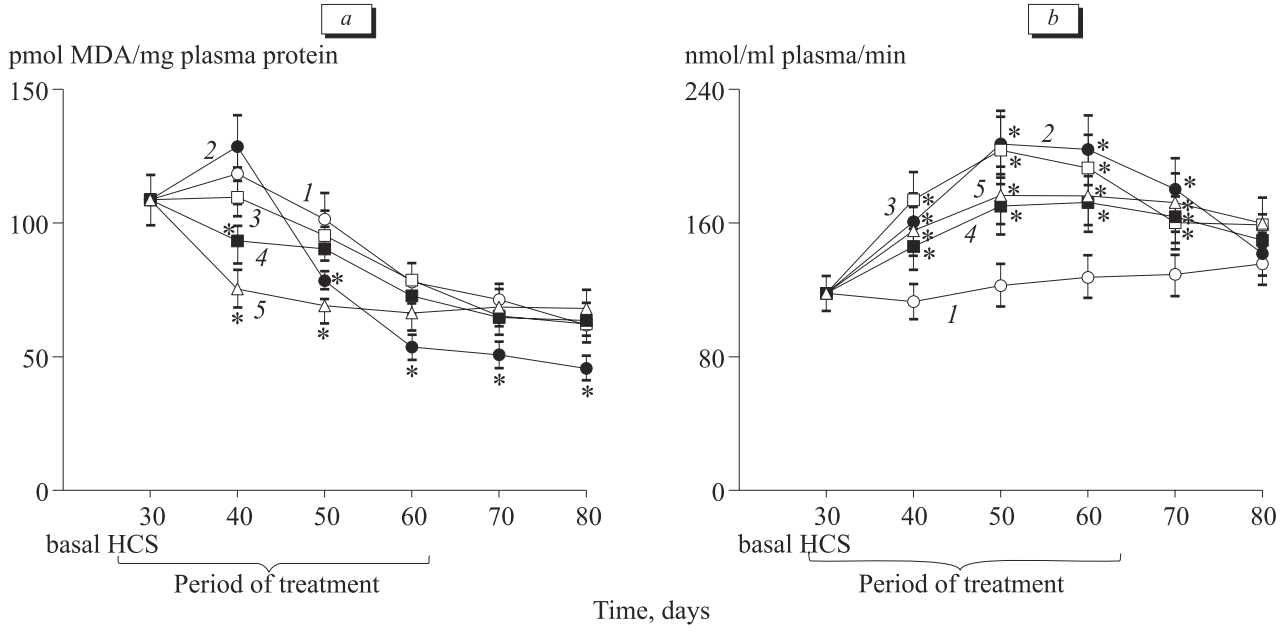

Fig. 1. Studying the antioxidant effect of SVG from variations in the content of LPO products (*a*) and paraoxonase activity (*b*) in the blood from rabbits with experimental HCS. Here and in Fig. 2: daily doses of SV and SVG. Control (*1*); 200 μg/kg SV (*2*); 1000 μg/kg SVG (100 μg/kg SV, *3*); 666 μg/kg SVG (66.6 μg/kg SV, *4*); and 400 μg/kg SVG (40 μg/kg SV, *5*). *$p<0.05$ compared to the control.

of treatment (40th day of the study), the content of LPO products in rabbits of the SVG group was below the control by 21 and 37%, respectively, and lower than in SV-treated animals by 27 and 41%, respectively ($p<0.05$).

Hence, the measurement of paraoxonase activity was of considerable importance. This antioxidant enzyme is associated with high-density lipoproteins. The interaction of paraoxonase with of low-density lipoprotein particles inhibits oxidative modification. Therefore, paraoxonase plays an important role in the pathogenesis of atherosclerosis [12]. Paraoxonase activity in SV-treated rabbits and animals of three-dose SVG groups (one-half, one-third, and one-fifth of SV) was higher compared to the control (by 42-60, 48-54, 29-39, and 37-45%, respectively, $p<0.05$), which attests to antioxidant activity of all studied doses of SVG (Fig. 1, *b*).

Possible effect of SVG on the endothelium was estimated from blood markers, including NO (major vasodilator continuously secreted by endothelial cells), von Willebrand factor (released from endothelial cells into the blood upon stimulation), and endothelin-1 (a factor not secreted by normal endothelial cells, but intensively produced by stimulated endotheliocytes) [11].

NO concentration in animals of the SV and SVG groups was higher than in control rabbits ($p<0.05$; Fig. 2, *a*). The increase in NO concentration was most pronounced after administration of SV (by 2 times compared to the control). A less significant increase in NO concentration was observed in SVG-treated animals (by 34-41, 49-51, and 59-75%, respectively, $p<0.05$).

The content of von Willebrand factor did not differ in rabbits of the SV and control groups (Fig.

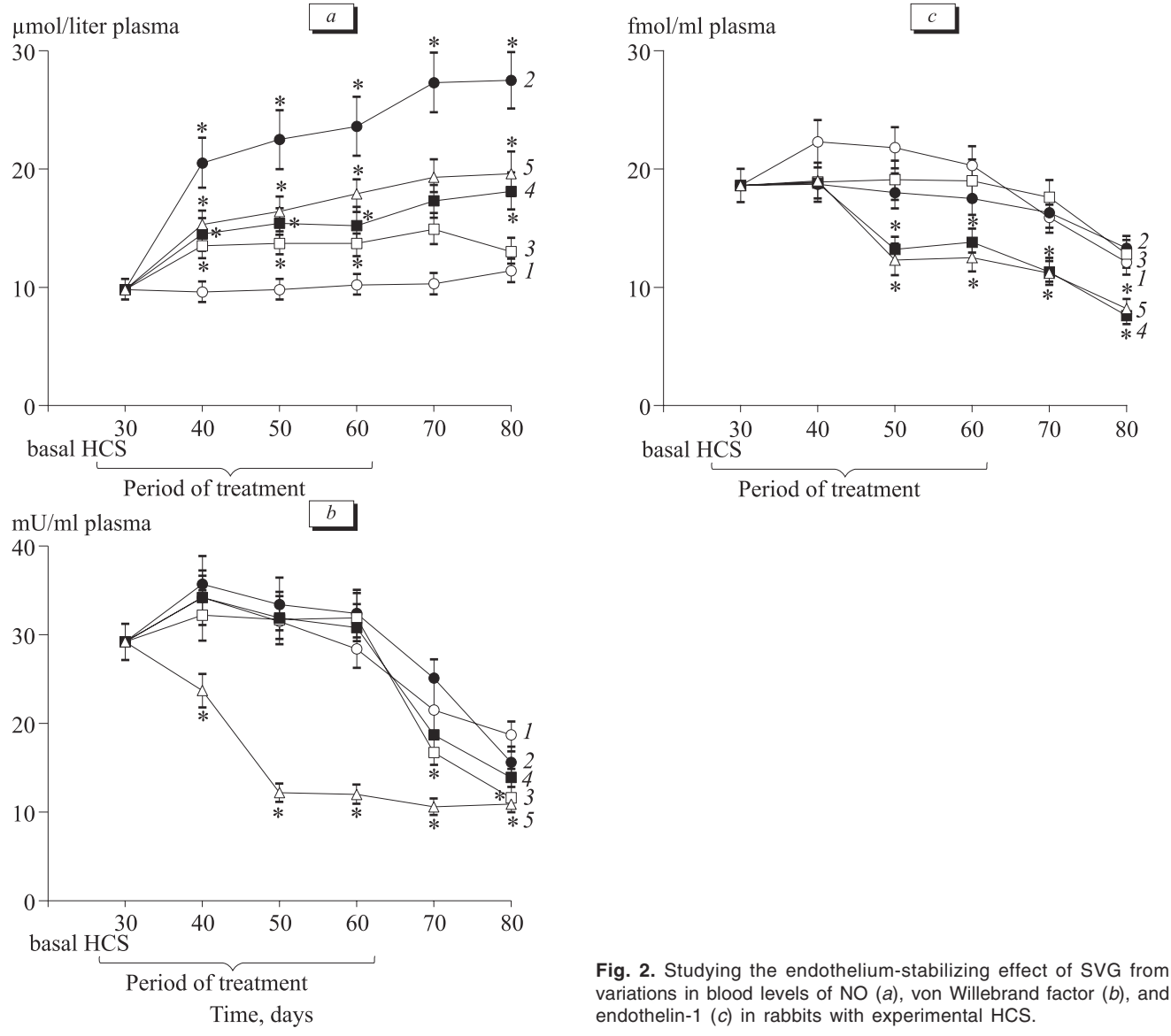

Fig. 2. Studying the endothelium-stabilizing effect of SVG from variations in blood levels of NO (*a*), von Willebrand factor (*b*), and endothelin-1 (*c*) in rabbits with experimental HCS.

2, *b*). On day 30, the level of von Willebrand factor in animals receiving SVG in doses of one-half and one-third of SV was lower compared to that in rabbits of the control and SV groups (by 22-26 and 26-33%, respectively, $p<0.05$). During treatment with the lowest dose of SVG and in the follow-up period, the level of von Willebrand factor was much lower than in the control and SV groups (by 51-61 and 58-64%, respectively, $p<0.05$).

Figure 2, *c* shows that SV and SVG (one-half of SV) had little effect on endothelin-1 concentration. Endothelin-1 concentration in rabbits decreased on days 20 and 30 of treatment with SVG in doses of one-third and one-fifth of SV (by 32-40 and 38-44%, respectively, compared to the control; and by 21-27 and 29-32%, respectively, compared to the SV group).

A negative correlation was found between the dose of SV and SVG and following effects: decrease in blood levels of LPO products (Pierson test, $r=-0.349$; Spearman test, $r=-0.317$; $p<0.01$), von Willebrand factor (Pierson test, $r=-0.289$; Spearman test, $r=-0.271$; $p<0.05$), and endothelin-1 (Pierson test, $r=-0.380$; Spearman test, $r=-0.351$; $p<0.01$) and increase in blood paraoxonase activity (Pierson test, $r=-0.237$; Spearman test, $r=-0.212$; $p<0.05$).

Thus, antioxidant activity of SVG is probably associated with the increase in blood paraoxonase activity. These changes are related to the fact that SVG increases high-density lipoprotein cholesterol level [4]. It reflects the increase in the amount of high-density lipoprotein particles carrying this enzyme. SVG produced a normalizing effect on endothelial function, which manifested in decreased blood levels of von Willebrand factor and endothelin-1. These effects were observed after administration of SVG in low doses (with respect to equivalent doses of SV). The dose dependence of the antioxidant and endothelium-stabilizing effect differed from the direct dose dependence of the hypocholesterolemic effect, which was revealed in our previous experiments with SVG [4]. These differences in dose dependences of the antioxidant, endothelium-stabilizing, and hypocholesterolemic effects are probably related to the fact that they involve various molecular targets. Hypocholesterolemic activity is associated with inhibition of HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis [1]. Studying the biological effects of chemical compounds showed that U-shaped curves and hormesis differ from a linear dose-effect relationship and are widely distributed [8]. However, little is known about these processes. The targets for endothelium-stabilizing activity remain unknown. However, our results suggest that this effect is achieved after treatment with a lower dose of test preparation (as compared to the cholesterol-reducing effect).

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