Study of Cholesterol-Lowering Effect and Safety of Simvaglisin on Rabbit Model of Hypercholesterolemia

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Using rabbit model of experimental hypercholesterolemia we showed that the hypocholesterolemic effect of simvaglisin, a complex preparation containing simvastatin and glycyrrhizic acid, in doses corresponding to 40, 66.5, and 100 mg/kg/day simvastatin is equal to the hypocholesterolemic effect of 200 mg/kg/day simvastatin alone. The total blood cholesterol decreased by 39, 36, 47, and 38% ($p<0.05$), respectively, after 20-day course of the preparation. Myotoxicity of simvaglisin evaluated by serum creatine phosphokinase was lower than that of simvastatin. After 30-day treatment, this parameter was lower by 26, 24, and 29% ($p<0.05$) than the corresponding parameter for simvastatin.

Key Words: *simvaglisin; simvastatin; hypercholesterolemia; lipid profile; liver enzymes*

Hypercholesterolemia plays an important role in the pathogenesis of atherosclerosis and CHD, highly prevalent pathologies associated with high mortality in Russia [2]. Treatment with cholesterol (CH)-lowering drugs is a priority strategy in the therapy of CHD and hypercholesterolemia [1]. Statins, inhibitors of hydroxymethylglutaryl-CoA reductase, are most effective in lowering LDL CH and reducing atherosclerosis- and CHD-associated mortality [6]. Intake of effective daily therapeutic dose of some statins determines the development of side effects: elevation of liver enzymes ALT and AST, myalgia, and myopathy with increased level of creatine phosphokinase [9]. Therefore, the search for new safer long-acting statins with lower daily dose effectively reducing CH content is in progress. A promissing approach is the combination of known pharmacons with natural complexons, *e.g.* glycyrrhizic acid. Potentiation of the drug effect compared to the initial preparations was demonstrated for the complexes of glycyrrhizic acid with phenylbutazone, indometacin [3], and nifedipine [5]. This approach was applied in the creation of simvaglisin (CVG), a new molecular complex of simvastatin (CV) with glycyrrhizic acid (1:4 stoichiometric ratio).

Here we evaluated CH-lowering potency and safety of CVG on *in vivo* rabbit experimental model of hypercholesterolemia.

MATERIALS AND METHODS

The experiment (80 days) was carried out on 25 male Gray Giant rabbits weighing 2.5-3.0 kg. The animals were maintained in individual cages and had free access to food and water. For induction of hypercholesterolemia, the animals fed a diet containing 5% animal fat and 3% CH (of total food volume) for 30 days [7,8]. Then the rabbits were divided into 5 groups and received hypocholesterolemic preparations for 30 days against the back-

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ground of normal diet: group 1 comprised control animals (intact); group 2 received 200 μg/kg/day CV; groups 3-5 received CVG in doses of 1000, 665 and 400 μg/kg/day, respectively. The weight ratio of CV in CVG is 0.1 of its molecular weight, and therefore the doses in groups 3-5 were below the dose of CV by 2, 3, and 5 times, respectively. The preparations were administered *per os* in a 1% starch suspension once a day. On days 61-80, the animals fed standard laboratory ration.

The blood (3 ml) was taken from the ear vein before and on days 30, 40, 50, 60, 70, and 80 of the experiment in the mornings. The animals were deprived of food 12 h before this procedure, but had free access to water.

Parameters of lipid profile of the serum (total CH, LDL CH, and tryglycerides) and activities of AST, ALT, and creatine phosphokinase (CK-Nac fraction) were measured on a Labsystem automatic biochemical analyzer using Biocon kits.

The data were processes statistically using Student test, correlation and dispersion analysis (One-Way ANOVA), and Dunnett test for multiple comparison. The differences were significant at $p<0.05$.

RESULTS

Feeding a CH-enriched diet during the first 30 days of the experiment led to the development of pronounced hypercholesterolemia (2309.0±223.2 mg/dl).

In the control group, the decrease in total CH content from the initial level became reliable on day 20 (35%, $p<0.01$). In experimental groups, significant decrease in total CH level was observed on day 10 (30-36%, $p<0.01$). The decrease in total CH was similar in different groups, despite the fact that the doses of CVG were lower by 2, 3, and 5 times than the dose of CV (Fig. 1). In all experimental groups, similar reliable decrease in total CH content by 36-47% was observed ($p<0.05$) after 20-day therapy with the test preparation, which attests to high CH-lowering activity of CVG (with consideration for 2-5-fold doses of CV in CVG). Thus, the effect of CVG surpassed that of CV.

The level of HDL CH in the control group remained unchanged throughout the experiment. In groups 2 and 3 it considerably increased by 25-29% ($p<0.05$) after 20-day treatment compared to the corresponding parameter on day 30 of the experiment (29.8±3.0 mg/dl), while after 30-day treatment it surpassed the control value by 20-26% ($p<0.05$). The test preparations had no effect on blood triglyceride content.

Apart from therapeutic hypocholesterolemic effect, statins produce some adverse effects, *e.g.*

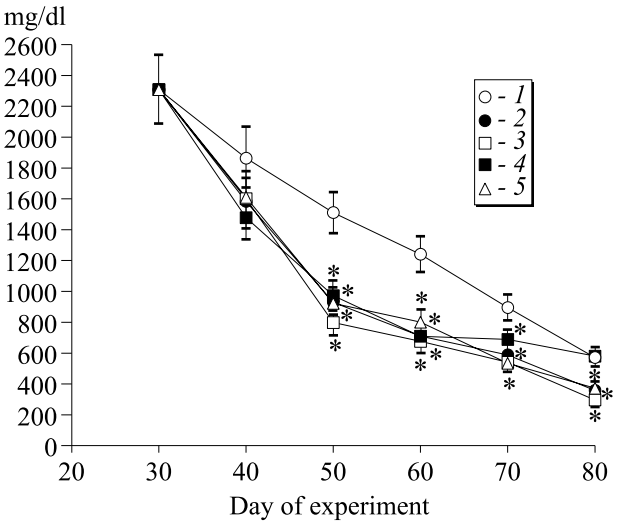

Fig. 1. Changes in total CH content in rabbits with experimental hypercholesterolemia treated with CVG. Here and on Figs. 2: *1-5*: groups 1-5; *$p<0.05$ compared to the control group.

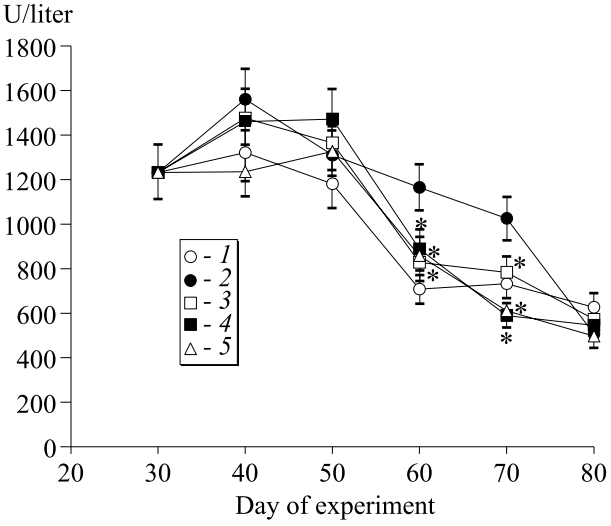

Fig. 2. Changes in creatine phosphokinase activity in the blood of rabbits with experimental hypercholesterolemia treated with CVG.

hepato- and myotoxicity. In rabbits receiving CV and CVG, plasma AST and ALT activities did not increase compared to the corresponding parameters on day 30 of the experiment (89.2±8.7 and 82.1±8.0 U/liter, respectively), which attested to the absence of the hepatotoxic effect of the specified doses of the test preparations. It should be noted that glycyrrhizic acid, a component of CVG, produces a hepatoprotective effect, in particular, it decreases transaminase activity and the content of lipoperoxides in hepatocytes.

Activity of creatine phosphokinase in the blood of group 2 rabbits increased as soon as on day 10 of therapy (by 27% compared to day 30 of the experiment, $p<0.01$); after 30-day course of CV this

parameter surpassed the control by 64%. In groups 3-5, this increase was less pronounced and insignificant compared to both the initial values and the corresponding parameters in the control group. On day 30 of treatment, this parameter in groups 3-5 was lower than in group 2 by 24-29% (Fig. 2). Hence, bearing in mind the content of CV in CVG we can conclude that both compounds produce similar myotoxic effects. However, more pronounced therapeutic effect ensure higher safety of CVG compared to CV.

Thus, CVG is a more effective and safe hypocholesterolemic preparation than CV. Our findings confirm our previous data [3,5] on the phenomenon of pharmacological synergism observed for combined use of some drugs with glycyrrhizic acid. In this case, we demonstrated the possibility of reducing the daily dose of CV in the complex preparation CVG, which diminished side effects without impairing drug efficiency.

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REFERENCES

1. Diagnostics and Correction of Lipid Metabolism Disturbances for Prophylaxis and Therapy of Atherosclerosis [in Russian], *Kardiovask, Ter, Profil.*, No. 2, Suppl. (2004).
2. R. G. Oganov and G. Ya. Maslennikov, *Ibid*., No. 3, 4-8 (2002).
3. G. A. Tolstikov, L. A. Baltina, E. E. Shults, and A. G. Pokrovskii, *Bioorg. Khimiya*, **23**, No. 9, 691-703 (1997).
4. G. A. Tolstikov, Yu. P. Nikitin, V. V. Lyakhovich, *et al.*, *Preparation with Hypolipidemic Effect Simvaglisin.* Patent application No. 2006105814. Priority from February 26, 2006. Patent decision April 2, 2007.
5. T. G. Tolstikova, I. V. Sorokina, A. O. Bryzgalov, *et al*., *Ratsionalnaya Farmakoterapiya in Kardiologii*, No. 1, 55-58 (2006).
6. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, *JAMA*, **285**, No. 19, 2486-2497 (2001).
7. T. Hayashi, P. J. A. Rani, A. Fukatsu, *et al*., *Atherosclerosis*, **176**, No. 2, 255-263 (2004).
8. F. Ishida, K. Watanabe, A. Sato, *et al., Biochim. Biophys. Acta*., Vol. **1042**, No. 3, 365-373 (1990).
9. P. Puddu, G. M. Puddu, and A. Muscari, *Acta Cardiol.*, **56**, No. 4, 225-231 (2001).