HYPOLIPIDEMIC AND ANTIOXIDANT ACTIVITY 
OF SULFUR-CONTAINING BISANIONS

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As is known, the drug probucol, which is used in the complex treatment of primary and secondary hyperlipidemia, reduces the total cholesterol (CS) level by changing primarily the content of low-density lipoproteins (LDL), while virtually not affecting triglycerides (TG) and very-low-density lipoproteins (VLDL). Despite insufficiently pronounced hypolipidemic activity, probucol favors a decrease in the necrosis zone in ischemized myocardium, inhibits plaque formation in the early stages of atherosclerosis, and restores the functional state of the antioxidant protection system [1, 2]. As for the chemical structure, probucol belongs to the group of sulfur-containing lipophilic bisphenols.

A distinctive feature of sulfur-containing compounds is the large breadth of therapeutic action. For example, unithiol and sodium thiosulfate produce detoxicating and anti-inflammatory effects, while peptides (camosine dipeptide and glutathione tripeptide), sulfur-containing amino acids (methionine, cysteine, and taurine), and sulfided aromatic compounds (both synthetic and natural) possess hypolipidemic, antiaggregant, cardiotropic, and antioxidant properties [3 - 6].

Investigations into the directed synthesis of biologically active sulfur-containing compounds, which have been extensively performed in recent years in the Research Institute of Experimental Medicine, include, in particular, the study of aromatic sulfonic acids (sulfobisanions) [7].

Our previous experiments on rabbits with models of experimental hyperlipidemia and atherosclerosis showed that one of the synthesized sulfobisanions (IEM-1064) exhibited combined hypolipidemic and antiatherosclerotic action, while another compound (IEM-1009) produced only an antiatherosclerotic effect [8]. Taking into account certain similarities in the chemical structure of sulfobisanions and probucol, and the antioxidant properties of the latter compound, we have suggested that the antiatherosclerotic activity of sulfobisanions can also be related to their antioxidant properties.

EXPERIMENTAL CHEMICAL PART

Sulfobisanions (compounds IEM-1009 and IEM-1064) were synthesized from the corresponding disulfodichloroanhydrides (diphenyl and diphenyl sulfide, respectively). Disodium salts of disulfodiphenyl (IEM-1009) and disulfodiphenyl sulfide (IEM-1064) were synthesized as described previously [7].

Structural formulas of the sulfobisanions studied and the reference compound (probucol) are as follows:

I (IEM-1009): 4,4'-biphenyldisulfonic acid disodium salt;
II (IEM-1064): 4,4-di(4-sulfoxyphenyl)sulfide disodium salt;
III (probucol): 4,4'-(isopropylidenedithio)-bis-(2,6-di-tert-butyl phenol).

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EXPERIMENTAL PHARMACOLOGICAL PART

The experiments were performed on white mongrel male rats weighing 200–220 g obtained from the Rappolovo nursery (Leningrad Region). The hyperlipidemia model in rats was induced by a cholesterol-rich diet as described in [9]. The total CS, TG, and the high-density lipoprotein (HDL) cholesterol (α-CS) in the blood serum was determined by fluorimetry using an automated Technicon AA-II system (USA). The α-CS concentration in the blood serum was measured upon precipitation of the apoB-containing lipoprotein fractions (VLDL and LDL). The CS content in the liver of experimental animals was determined using the Liebermann–Burchard color reaction, and the TG content was measured as described in [10]. The products of lipid peroxidation (LPO) in the blood serum of rats were determined fluorometrically [11].

The test animals were divided into several groups receiving different diets:

Group 1 obtained the basic laboratory ration (control group);

Group 2 received a hypercholesteremic (HCS) diet including 3% CS, 0.12% 6-methyluracyl, and 30% of heated fat mixture (sunflower oil – pork fat);

Group 3: HCS diet with IEM-1009 (200 mg/kg, p.o.);

Group 4: HCS diet with IEM-1064 (200 mg/kg, p.o.);

Group 5: GCS diet with probucol (100 mg/kg, p.o.).

The experiment lasted for 21 day.

As is known, heparin and some sulfated polysaccharides can interact with apoB-containing lipoproteins in vitro [12] and prevent their penetration into arterial walls, thus inhibiting the development of atherosclerosis [13, 14]. Therefore, it was important to check whether the sulfobisanions studied in this work possess such properties. The ability of binding atherogenic lipoproteins was studied by a turbidimetric method previously used to compare the efficacy of binding such lipoproteins by various polar polysaccharides [9]. The lipo-protein binding tests were performed with a hyperlipidemic human blood serum (CS content, 250 mg/dl) and 1% solutions of the sulfobisanions studied. The reference compound was heparin at the same concentration, for which the binding efficacy was taken equal to 100%.

The antioxidant properties of sulfobisanions IEM-1009 and IEM-1064 were evaluated by their effect on the content of fluorescent LPO products in rat blood serum under the experimental hyperlipidemia conditions.

The experimental data were statistically processes using the Student t-criterion.

RESULTS AND DISCUSSION

As seen from Table 1, prolonged administration of a cholesterol-rich diet results in a twofold increase in the CS content in the blood serum of rats and in a 4.5-fold increase in the levels of CS and TG in the rat liver. At the same time, the TG level in the blood serum of test rats is reliably below that in the control group, which is related to the presence of a thyroid suppressor – 6-methyluracyl – without which it would be difficult to induce hyperlipidemia in animals of the species studied. Note also a significant decrease in the content of α-CS in the blood serum.

In the group of animals treated with IEM-1064, the content of CS decreases both in the blood serum (27%) and in the liver (40%). The experimental animals treated with IEM-1009 show a reliable decrease in CS in the blood serum (26%) and a tendency toward decreasing CS in the liver. Probucol also exhibits a reliable hypocholesteremic effect and reduces the CS content in rat liver.

Thus, the experiments on rats with induced hyperlipidemia model showed that sulfobisanion IEM-1064 produces a reliable hypolipidemic action, which is more pronounced as compared to that of IEM-1009 and comparable with that of probucol.

<table>
<thead>
<tr>
<th>Experimental Part</th>
<th>Blood serum</th>
<th>Liver</th>
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<tbody>
<tr>
<td></td>
<td>CS, mg/dl</td>
<td>α-CS, mg/dl</td>
</tr>
<tr>
<td>1 Intact rats (n = 8)</td>
<td>71.5 ± 5.7</td>
<td>41.2 ± 2.5</td>
</tr>
<tr>
<td>2 HCS diet (n = 11)</td>
<td>137.2 ± 5.2</td>
<td>22.8 ± 2.7</td>
</tr>
<tr>
<td>P2 &lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>3 HCS diet + IEM-1009 (n = 11)</td>
<td>100.0 ± 5.5</td>
<td>22.3 ± 2.3</td>
</tr>
<tr>
<td>P3 &lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>4 HCS diet + IEM-1064 (n = 11)</td>
<td>97.0 ± 3.7</td>
<td>23.5 ± 2.3</td>
</tr>
<tr>
<td>P4 &lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>5 HCS diet + probucol (n = 13)</td>
<td>86.4 ± 7.3</td>
<td>25.2 ± 1.9</td>
</tr>
<tr>
<td>P5 &lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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* n indicates the number of animals in the test group.