2. A. Wentzel, *Theorie zufalliger Progresse*, Birkhauser (1979).
3. L. I. Ivanova and E. M. Khalilov, *Biofizika*, № 1, 156-158 (1986).
4. V. G. Ivashkin, V. Yu. Vasil'ev, and E. S. Severin, Levels of Regulation of the Functional Activity of Organs and Tissues [in Russian], Leningrad (1987), p. 92.
5. B. S. Kasavina, T. V. Ukhina, and T. K. Demina, *Dokl. Akad. Nauk SSSR*, **222**, № 1, 136-139 (1975).
6. E. P. Frolov (Ed.), *The Skin: Structure, Functions, General Pathology, and Therapy* [in Russian], Moscow (1982), p. 123.
7. E. I. Kreps, *Lipids of Biological Membranes* [in Russian], Leningrad (1979).
8. P. V. Sergeev, *Steroid Hormones* [in Russian], Moscow (1984), pp. 116-197.
9. Yu. K. Skripkin, *Skin and Venereal Diseases* [in Russian], Moscow (1979).
10. J. Tepperman and H. Tepperman, *Metabolic and Endocrine Physiology*, Year Book Medical Publishers, Inc., Chicago-London (1987).
11. A. White, P. Handler, E. Smith, *et al.*, *Principles of Biochemistry*, McGraw-Hill, New York (1978).
12. W. Bachman, E. M. Merisko, G. K. Ojakian, *et al.*, *J. Biol. Chem.*, **256**, 982-988 (1981).
13. R. V. Farese, *Molec. Cell. Endocr.*, **35**, № 1, 1-14 (1978).
14. J. Folch, M. Lees, and S. G. H. Staane, *J. Biol. Chem.* **226**, 497-509 (1957).
15. E. Harms, *Biochem. J.*, **166**, № 3, 455-462 (1977).
16. G. Leonardi, C. Schneider, and A. Neufahrt, *Therapiewoche*, № 38, 6168-6177 (1980).
17. H. Saiton, K. Nirato, and R. Tahara, *Acta Endocr. (Kbh.)*, **10**, № 3, 420-424 (1984).
18. W. B. Weglicki, *Biochem. Biophys. Res. Commun.*, **124**, № 1, 229-235 (1984).

Effect of Cimetidine on Experimental Atherogenesis in Rabbits

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Cimetidine is an antiulcer drug that blocks the H_2-receptors and inhibits cytochrome P-450 in the liver. It is known that a low activity of liver monooxidases promotes damage to the intima in experimental atherosclerosis in rabbits, whereas P-450 induction has an antiatherogenic effect [2,10]. On the other hand, antioxidative properties of cimetidine have recently been discovered [14], which may be important for atherogenesis inhibition.

In the present study the effect of cimetidine on experimental atherogenesis in rabbits was investigated.

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MATERIALS AND METHODS

The experiments were carried out on 27 male chinchilla gray rabbits weighing 2.1-2.9 kg. Experimental atherosclerosis was modeled by daily (6 times a week) feeding of a 10% cholesterol solution in sunflower oil (200 mg/kg) through gastric intubation during 12 weeks. All rabbits were divided into 3 groups, with 9 animals in each. The first group consisted of control animals which received sunflower oil (2 ml/kg) through a gastric tube in addition to standard laboratory chow. The second group received the cholesterol solution in sunflower oil. The third group received 10 mg/kg cimetidine (histodyl, Gedeon Richter, Hungary) in a 1% starch suspension through a gastric tube together with cholesterol. Blood for

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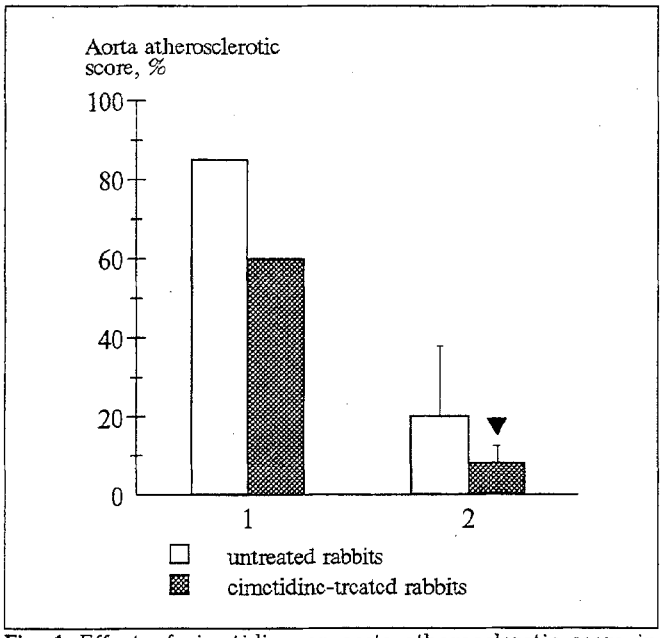

Fig. 1. Effect of cimetidine on aorta atherosclerotic score in rabbits. Abscissa: 1) aorta involved in lipidosis; 2) aorta atherosclerotic score; triangles: the difference is statistically reliable.

biochemical study was drawn monthly from the marginal ear vein after an 18-hour fast. Twelve weeks after the beginning of the experiment the rabbits were killed by embolization. Weighted samples of the liver, myocardium, and aorta wall intima-media were frozen in liquid nitrogen and then homogenized for malonic dialdehyde (MDA) determination using the thiobarbituric-acid-reactive substances assay [5]. The liver lipids were extracted with chloroform-methanol 2:1 by the Folch method [9] and analyzed by thin-layer chromatography (TLC) on Silufol plates (Kavalier, Czechoslovakia). The content of total lipids (TL) and the following lipid fractions were determined: free cholesterol (FCh), cholesterol esters (ECh), triglycerides (TG), and phospholipids (PL). The FCh/PL and FCh/TCh ratios were calculated. The TCh content in the blood serum was determined as described [3], the concentration of high-density lipoprotein cholesterol (HDL-Ch) was assessed by heparin-$MgCl_2$ precipitation [7], the TL and TG concentrations were estimated with "Lachema" diagnostic kits (Czechoslovakia), PL was estimated as described [13], and MDA as mentioned above; the atherogenicity coefficient was calculated according to formulas [8]. Erythrocytes were separated for determination of peroxidative hemolysis [4]. A planimetry of the aorta wall [1] and morphometry of the liver were carried out. The specimens were stained with hematoxylin and eosin, and the lipids were contrasted with Sudan III. The results were processed statistically using the Student t test.

RESULTS

The planimetry of the aorta wall revealed that in 6 out of 7 "untreated " animals intima lesions such as lipid deposits were observed. Aorta intima lipidosis was found only in 4 out of 7 rabbits treated with cimetidine, whereas in 3 others the intima was unaffected. Cimetidine markedly decreased the aorta atherosclerotic score: 5.0±2.3% in cimetidine-treated animals as against 22.5±8.4% in untreated ones (see Fig.1).

In parallel with the antiatherosclerotic effect cimetidine reduced lipid infiltration in the liver. From TLC data, 12-week cholesterol feeding led to an almost threefold elevation of the TG level in the liver, and the ECh content was increased by 6, TG by 2.5, and PL by 2 times. The protective effect of cimetidine on the liver manifested itself mainly as inhibition of ECh accumulation. The biochemical data were confirmed by a morphological examination. The pattern of lipid dystrophy was seen to change from large-drop (50% in untreated animals vs. 16.7% in cimetidine-treated ones) to small-drop

TABLE 1. Effect of Cimetidine on Content Lipid Fractions in the Liver of Cholesterol–Fed Rabbits 12 Weeks after Hypercholesterolemia, mg/g Wet Tissue

Parameter	Group of animals				
	control (n=9) $M\pm m$	hypercholesterolemia (n=9)		hypercholesterolemia+cimetidine (n=9)	
		$M\pm$m	P_1	$M\pm$m	P_2
TL	17.27±0.30	49.01±4.55	<0.001	30.71±1.80	<0.01
TCh	4.16±1.2	20.49±3.05	<0.001	10.08±0.86	<0.01
FCh	1.03±0.47	3.01±0.35	<0.01	2.49±0.19	>0.05
ECh	3.13±0.77	17.78±2.79	<0.001	7.59±0.69	<0.01
FCh/TCh	0.23±0.04	0.15±0.015	<0.05	0.25±0.007	<0.05
PL	7.81±0.91	15.22±1.44	<0.05	11.17±1.06	<0.05
FCh/PL	0.14±0.077	0.21±0.027	>0.05	0.23±0.022	>0.05
TG	5.30±0.03	13.03±1.36	<0.01	9.46±1.42	<0.05

Note: here and in Table 2: P_1 is the difference between the second and the first group; P_2 is the difference between the third and the second group.

TABLE 2. Effect of Cimetidine on Some Indexes of Lipid Peroxidation in Rabbits at 12 Weeks of Hypercholesterolemia Development.

Parameter	Group of animals				
	control (n=9) $M\pm m$	hypercholesterolemia (n=9)		hypercholesterolemia+cimetidine (n=9)	
		$M\pm m$	P_1	$M\pm m$	P_2
MDA, blood, nmol/liter	3.92±0.16	8.24±0.22	<0.05	4.36±0.22	<0.05
Peroxidative hemolysis of erythrocytes, blood, %	3.03±0.21	23.01±1.88	<0.001	7.36±0.76	<0.01
MDA, aorta, nmol/g wet tissue	13.82±1.21	20.00±2.00	<0.05	15.33±1.57	<0.05
MDA, liver, nmol/g wet tissue	17.65±1.98	25.75±2.30	<0.05	20.30±4.90	>0.05
MDA, heart, nmol/g wet tissue	15.80±2.60	21.99±2.97	>0.05	17.87±3.86	>0.05

(50% vs. 88.3%, respectively). A small-drop pattern is to be considered as more benign.

Remarkably, the antiatherosclerotic and hepatoprotective effects of cimetidine are realized without any hypolipidemic action. The study of the main lipid fractions demonstrated that 12 months after the experiment started TCh was 2.24±0.15 mM/liter in the control group, 12.2±1.64 mM/liter in the hypercholesterolemic group, and 15.07±1.97 mM/liter in the cimetidine-treated group. Correspondingly, the blood TG level was 1.83±0.11 mM/liter in the control, 4.79±0.64 mM/liter in hypercholesterolemia, and 4.58±0.55 mM/liter after cimetidine treatment. No significant influence of cimetidine on the LDL-Ch to HDL-Ch ratio or total PL level was detected either. At the same time, there are data to the effect that cimetidine can increase the HDL-Ch concentration in CHD patients. This effect, however, is reported to be accompanied by severe hyperchylomicronemia [11].

An attempt was undertaken to connect the antiatherogenic and hepatoprotective properties of cimetidine with its influence on lipid peroxidation. The results are presented in Table 2. It was shown that cimetidine normalized both the processes of peroxidative erythrocyte hemolysis and the level of MDA in the blood. A significant drop in the MDA concentration in the aorta wall was also observed. Co-directed shifts were detected in the heart and liver tissues, but they were not statistically reliable. The data suggest that the antiatherogenic effects of cimetidine are realized through an inhibition of lipid peroxidation.

Our assumption is confirmed by the data of Uchida *et al.* [12], who demonstrated that cimetidine exerts a strong and multidirectional influence on different steps of free-radical processes: for instance, it binds hydroxyl radicals and copper II ions; it neutralizes hydrogen peroxide and lipid peroxides; the cimetidine-Cu II complex exhibits pronounced superoxide dismutase activity.

It was also shown that H_2-receptors blocked by cimetidine may decrease the permeability of the wall of the cerebral vessels [6].

Nevertheless, the mechanism of the antiatherogenic action of cimetidine remains incompletely understood. Nor is it clear whether the inhibitory influence of cimetidine on the liver microsomal enzymes is involved in hepatoprotection in hypercholesterolemia or whether these effects are independent.

We believe that the antiperoxide properties of cimetidine may play a substantial role for protection of the liver and vessel wall against experimental atherosclerosis.

REFERENCES

1. G. G. Avtandilov, *Classification and Planimetric Evaluation of Vascular Atherosclerotic Lesions* [in Russian], Nalchik (1980).
2. G. N. Antonova, E. M. Khalilov, G. I. Bachmanova, *et al., Vopr.Med.Khimii*, **30**, № 2, 67 (1984).
3. E. Yu. Gerasimova, Manual of Cardiology [in Russian], Moscow (1982), Vol. 2, p. 532.
4. A. A. Pokrovskii, A. A. Abrarov, *Vopr. Pitaniya*, **23**, № 2, 47 (1964).
5. I. D. Stal'naya, *Modern Methods in Biochemistry* [in Russian], Moscow (1977), p. 63.
6. S. B. Boertje, D. Le Beau, and C. Williams, *Neuropharmacology*, **28**, 749 (1989).
7. M. Burstein, H. R. Scholnick, and R. Morfin, *J. Lipid Res.*, **11**, 583 (1979).
8. W. T. Friedewald, R. I. Levy, and D. S. Fredrikson, *Clin. Chem*, **18**, 499 (1972).
9. M. Kates, *Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids*, Amsterdam (1986).
10. P. V. Luoma, A. Rautio, I. Stengard, *et al., Europ. J. Clin. Pharmacol.*, **38**, 625 (1990).
11. G. R.Thompson, *A Handbook of Hyperlipidaemia*, London (1990).
12. K. Uchida and S. Kawakishi, *Agricult. Biol. Chem.* **54**, 2485 (1990).
13. A. Zilversmit and A. Davis, *J. Lab. Clin. Med.*, **36**, 155 (1950).
14. J. Zimmerman and J. Millard, *Clin. Pharmacol. Ther.*, **45**., 487 (1989)