Comparative Characteristics of the Antiatherogenic Effect of Xymedon and Parmidin

R. A. Kamburg, O. B. Ibragimov, M. B. Kondrat'eva, I. Kh. Valeeva, and N. Sh. Shamsutdinov

UDC 615.272.4.038

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 8, pp. 205-208, August, 1993
Original article submitted March 24, 1993

Key Words: *xymedon; parmidin; experimental atherosclerosis*

In our search for new drugs possessing a direct antiatherosclerotic effect we turned to xymedon. Xymedon is a heterocyclic nitrogen-containing compound and a pyrimidine derivative. In clinical studies the efficacy of the drug as a stimulator of post-burn regeneration has been proven [2,4]. Previously, using the experimental model of local cryodestruction, we established the ability of xymedon to promote repair processes in the endothelial lining [10]. In experimental studies xymedon was found to inhibit the accumulation of total lipids (TL) and cholesterol (CH) in the aorta and liver in rats and to reduce the content of lipoperoxides in these organs. In blood serum the drug was discovered to normalize the metabolism of lipids and lipoproteins only in the early stages of hypercholesterolemia in rats [9]. The aim of the present study was to compare the effects of xymedon and an other antiatherosclerotic drug, parmidin, using the model of experimental hypercholesterolemia in rabbits.

MATERIALS AND METHODS

The experiments were conducted on 36 male gray chinchilla rabbits with an initial weight of 2.1-2.9 kg. Experimental atherosclerosis was induced by daily feeding through gastric intubation a 10% CH solution in sunflower oil (200 mg/kg) during 12 weeks. The rabbits were divided into 4 groups with 9 animals in each. The first, control, group received sunflower oil (2 ml/kg) through a gastric tube along with standard laboratory chow. The second group received the CH solution in sunflower oil. The third group received xymedon (the xymedon preparation was synthesized by A. E. Arbuzov at the Research Institute of General and Physical Chemistry, and was kindly supplied by V. S. Reznik) in a dose of 30 mg/kg in a 1% starch suspension through a gastric tube together with CH. The fourth group received parmidin (the parmidin preparation (pyridine carbomate) was kindly supplied by Gedeon Richter, Hungary) in a dose of 30 mg/kg by the same route. Blood for biochemical study was drawn from the marginal ear vein after an 18-hour fast after 4 and 12 weeks of the experiment. Twelve weeks after the beginning of the experiment the rabbits were killed by embolization. The liver lipids were analyzed by thin-layer chromatography on Silufol plates (Kavalier, Czechoslovakia). Weighted samples of the liver were homogenized and lipids were extracted with chloroform-methanol 2:1 after Folch [8]. The content of TL and the following lipid fractions was determined: free cholesterol (FCH), cholesterol esters (ECH), triglycerides (TG), and phospholipids (PL). The content of total CH (TCH=FCH+ECH) and the TCH/PL coefficient were calculated. The content of TCH, CH of high-density lipoproteins (HDL-CH), and TG was measured with a Labsystems

Central Research Laboratory, Department of Pathological Anatomy, Kazan' Medical Institute. (Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0008-1017$12.50

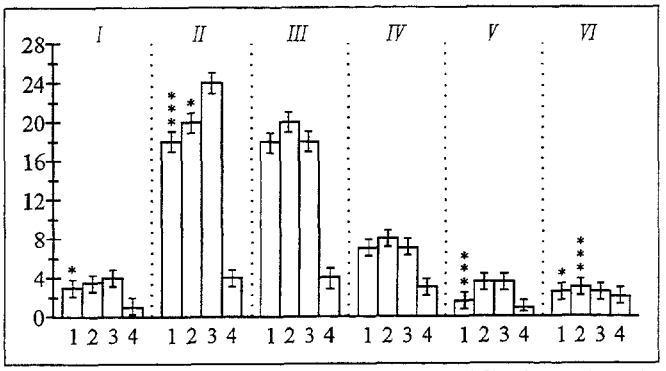

Fig. 1. Effect of xymedon and parmidin of lipid fractions in rabbit liver after 12 weeks of hypercholesterolemia (mg/g wet tissue). Applicate groups: *1*) control; *2*) hypercholesterolemia; *3*) hypercholesterolemia+xymedon; *4*) hypercholesterolemia +parmidin; lipid fractions: *I*) free cholesterol (FCH), *II*) cholesterol esters (ECH), *III*) triglycerides (TG), *IV*) phospholipids (PL), *V*) free fatty acids (FFA), *VI*) total cholesterol to phospholipid ratio (TCH/PL); ordinate: lipid fraction content (mg/g wet tissue).

autoanalyzer (Finland) using diagnostic kits; PL were estimated by phosphorus content [13]; malonic dialdehyde (MDA) was measured using the thiobarbituric-acid-reactive substances assay [7]. The atherogenicity coefficient was calculated according to the formula: [TCH-HDL-CH]/TCH. The aorta atherosclerotic score (AAS) was determined by computer planimetry. Lesions involving no more than 15% of the aorta surface were considered minimal. Morphological study of the aorta and liver was performed after staining of the specimens with hematoxylin and eosin, toluidine blue, and Sudan III. The data obtained were processed statistically using the Student *t* test and the nonparametric Wilcoxon-Mann-Witney *U* test [3].

RESULTS

After 4 weeks of hypercholesterolemia marked proatherogenic changes were observed in the serum of "untreated" animals: a dramatic rise of the TCH content and 2-fold rise of the atherogenicity coefficient (Table 1). Xymedon exhibited no hypocholesterolemic effect; however, in comparison with the "untreated" group, it substantially lowered the atherogenicity coefficient due to a 75% increase in HDL-CH. In the parmidin-treated group no changes in the dynamics of lipid metabolism were observed. During the 12th week of CH feeding the proatherogenic alterations were further exacerbated. In the xymedon-treated group a mild hypolipidemic effect of the drug was observed; parmidin also lowered the level of TCH in the blood: 12.6±0.41 mmole/liter in hypercholesterolemia, but 9.6±1.74 mmole/liter and 10.9±0.36 mmole/liter under the influence of xymedon and parmidin, respectively ($p<0.001$).

In hypercholesterolemia over the entire experiment an almost 3-fold increase of the content of the end product of lipoperoxidation, MDA, was observed (Table 1). Xymedon treatment was accompanied by a reliable drop of this index. Parmidin decreased the content of MDA only at early stages of hypercholesterolemia.

Chromatography of the liver lipids is presented in Fig. 1. The 12-week CH feeding resulted in a 4-fold increase in TL, mainly due to elevation of ECH and FCH (by 5 and 4.2 times, respectively). The content of TG rose by 3.5 and of PL and FFA by 3 times, and the TCH/PL ratio was also increased. Treatment with both xymedon and parmidin reduced the content of TCH in the liver from 28.2±3.9 mg/g in hypercholesterolemia to 21.6±5.7 mg/g ($p<0.05$) under the influence of xymedon and to 19.1±2.0 mg/g ($p<0.001$) under the influence of parmidin. The level of TG and PL did not change reliably, but both drugs, and especially xymedon, lowered the TCH/PL ratio. The biochemical data relating to the liver were confirmed by the morphological findings. The pattern of lipid dystrophy was seen to change from large-drop to a more benign small-drop. The ability of xymedon to preserve the liver from lipid

TABLE 1. Effect of Xymedon and Parmidin on Lipid Metabolism and the Content of MDA in Rabbit Serum after Four Weeks of Hypercholesterolemia ($M\pm m$, $n=9$)

Index	Group of animals			
	Control	Hypercholesterolemia	Hypercholesterolemia+xymedon	Hypercholesterolemia+parmidin
TCH, mM	1.56±0.42	4.37±0.22	5.90±0.49*	4.39±0.49
HDL−CH, mM	0.46±0.06	0.91±0.25	1.59±0.17*	0.86±0.15
TG, mM	2.21±0.14	1.05±0.19	2.23±0.46*	1.50±0.33
PL, mM	0.94±0.08	1.31±0.26	1.79±0.33	1.37±0.18
Atherogenicity coefficient	2.28±0.42	4.60±0.81	2.83±0.99*	3.96±0.92
MDA, nM	0.32±0.06	1.41±0.06	1.12±0.11*	1.11±0.13*

Note. *p* is the difference between "treated" and "untreated" animals; *: $p<0.05$.

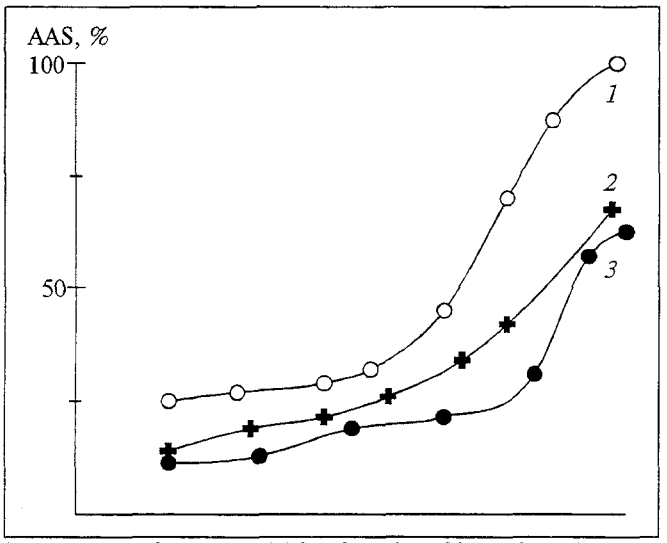

Fig. 2. Distribution of AAS values in rabbits after 12 weeks of hypercholesterolemia under the influence of xymedon and parmidin. Abscissa: values of AAS for the following groups: *1*) control; *2*) hypercholesterolemia; *3*) hypercholesterolemia + xymedon; *4*) hypercholesterolemia + parmidin.

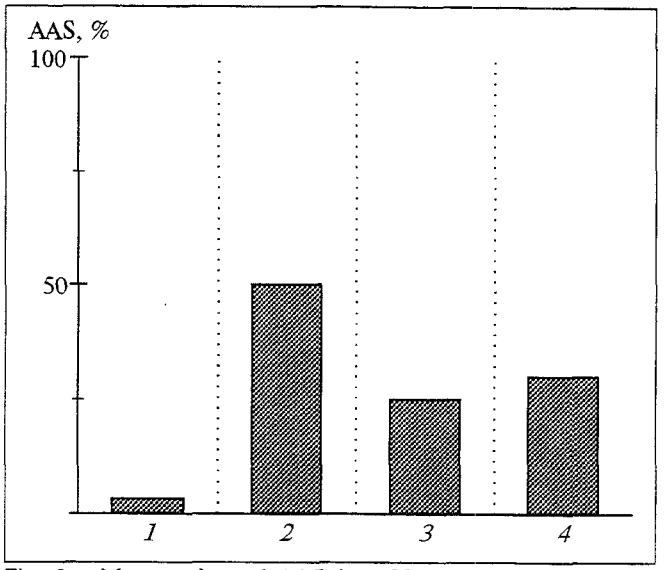

Fig. 3. Mean value of AAS in rabbits after 12 weeks of hypercholesterolemia under the influence of xymedon and parmidin. Abscissa: mean values of AAS for the following groups: *1*) control; *2*) hypercholesterolemia; *3*) hypercholesterolemia + xymedon; *4*) hypercholesterolemia + parmidin.

dystrophy is in conformity with our previous results, obtained with the model of experimental hypercholesterolemia in rats [9].

Planimetry of the aorta wall revealed (Figs. 2 and 3) that all "untreated" animals had intima lipidosis: the AAS was 17.6% to 96,7%, being 44.9% on average. Out of 6 xymedon-treated rabbits in 4 animals the lesions of the intima were minimal, and only in 2 rabbits was intima lipidosis observed; the AAS was 0% to 72.7%, being 24.1% on average. In the parmidin-treated group the intima had minor lesions in 3 animals and in 4 animals intima lipidosis was found; the AAS was 2% to 70.5%, being 26.1% on average. The statistical analysis with the use of the nonparametric U test revealed a reliable ($p<0.05$) decrease of the AAS in the xymedon-treated group in comparison with the hypercholesterolemic group. The differences for the parmidin-treated group were insignificant. Morphological study revealed markedly irregular thickenings of the aorta intima in "untreated" animals and basophilia and metachromasia of the intima. Lipid deposits were seen in both the intima itself and the adjacent media. In rabbits with extensive lesions of the aorta the intima was represented by foam cells, which in some places formed plaque-like structures. In the xymedon-treated animals the intima stained with toluidine blue was intact, while Sudan III staining revealed a diffuse dustlike infiltration of the intima, without lipid deposits being found in the media. Cell proliferation and the presence of foam cells were not noted in the intima. In the parmidin-treated group we observed a similar morphological picture; however, in some cases irregular microthickenings of the intima, plaque-like structures, and the accumulation of foam cells were seen.

The data obtained allow us to conclude that in rabbits with experimental atherosclerosis xymedon exhibits pronounced antiatherosclerotic and angioprotective properties. The efficacy of the drug was comparable with that of parmidin, which was used as a reference drug. The effects of the latter were found to be consistent with published data [5,11,12]. Unlike the classic angioprotector, xymedon was shown in the present and previous studies to normalize lipoprotein metabolism by elevating HDL-CH, to promote regeneration of damaged endothelium, and to possess excellent immunomodulatory properties, which may be of importance for the realization of its antiatherosclerotic effect. Taking into account the low toxicity and sufficient tolerance in clinical application, it may be stated that the possibilities for the clinical use of xymedon as an antiatherosclerotic remedy are very promising.

REFERENCES

1. G. G. Avtandilov, *Classification and Planimetric Evaluation of Atherosclerotic LesionS of Blood Vessels* [in Russian], Nal'chik (1980).
2. S. M. Gorbunov, *Pharmacological and Toxicological Characteristics of Xymedon and Its Effect on Thermal Skin Burns.* Author's abstract of Ph. D dissertation [in Russian], (1979).
3. E. V. Gubler, *Computative Methods of Recognizing Pathological Processes* [in Russian], Leningrad (1970).
4. O. S. Kochnev and S. G. Izmailov, *Kazan. Med. Zh.*, 71, № 5, 373-375 (1990).

5. V. E. Ryzhenkov, E. M. Tararak, G. G. Khechinashvili, *et al.*, *Byull. Eksp. Biol. Med.*, **81**, № 5, 558-559 (1976).
6. Yu. D. Slabnov, *Experimental Substantiation of the Use of Xymedon as an Immunomodulator.* Author's Abstract of M.D. dissertation [in Russian], Kazan' (1988).
7. *Current Methods in Biochemistry*, Ed. V. N. Orekhovich [in Russian], Moscow (1977).
8. J. M. Folch and G. A. Sloane-Stanley, *J. Biol. Chem.*, **226**, 497 (1957).
9. R. Kamburg, L. Popova, and O. Ibragimov, in: *59th International Atherosclerosis Society Congress*, Nice (1992), p. 120.
10. R. Kamburg, O. Ibragimov, and A. Mironov, *Acta Physiol. Scand.*, **146**, Suppl. 608, 174 (1992).
11. T. Shimamoto, *Acta Path. Jap.*, **19**, 15 (1969).
12. C. C. Wu, T. S. Huang, and C. J. Hsu., *Amer. Heart J.*, **77**, 657 (1969).
13. A. Zilversmit and A. Davis, *J. Lab. Clin. Med.*, **36**, 155 (1950).

Morphological Mechanism of the Development of Miosatellitocytes from Structural Elements of Muscle Fiber under Conditions of Increased Functional Activity of Skeletal Muscles

B. M. Mytskan, V. B. Shutka, V. A. Shakhlamov, and M. A. Mytskan

UDC 612.741.014.2.08

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 8, pp. 208-210, August, 1993
Original article submitted March 30, 1993

Key Words: *development of myosatellitocytes; muscle fiber; functional activity*

As is commonly known, myosatellitocytes (MSC) develop from promyoblasts, the sole source of development of muscle tissue [1]. The appearance of mature MSC is preceded by structural-metabolic transformations of the initial cells, which take place simultaneously with the development of muscle fibers (MF). The final differentiation occurs as the muscle matures as an organ, after which the MSC are retained under the basal membrane of the MF [6]. Under extreme conditions of the development of muscle tissue (regeneration after injury, atrophy, circulatory disorders in the muscle, denervation, hypertrophy), MSC act as the muscular cambium [8,12-14]. Moving into the interstitial space, MSC are transformed into regenerative myoblasts [5]. It has been shown that MSC can become part of MF [6]. Their cytoplasm thereupon merges with the sarcoplasm of the MF, while the nuclei of the incorporated MSC, losing the capacity for proliferation, become component elements of the myosymplast.

A hypothesis of discontinuity, or the formation of the cellular phase of myogenic tissue from the acellular (myosymplastic) phase, has been put forward [3,9,10,15]. Several investigations have been carried out to verify this hypothesis [4,7,10]. The results reported, however, are not very convincing. They seem to prove not the process of new formation of MSC, but rather different stages of ejection of satellite cells from MF or their incorporation into the myosymplasts. On the other hand, it is not to be excluded that a mechanism exists for the new formation of MSC from components

Department of Human Anatomy, Ivano-Franko Medical Institute; Laboratory of Experimental Cell Pathology, Institute of Human Morphology, Russian Academy of Medical Sciences, Moscow. (Presented by N. K. Permyakov, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0008-1020$12.50