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PATHOMORPHOLOGICAL CRITERIA OF THE EXPERIMENTAL ANTIATHEROSCLEROTIC ACTION OF SAPONINS OF PLANT ORIGIN

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Regression of atherosclerosis is observed during its treatment by preparations such as clofibrate, lipostabil, Adriamycin, diethylhexylphthalate, and so on [10, 12-14]. However, their long-term administration runs the risk of toxico-allergic side effects. In recent years attention has been drawn to preparations of plant origin, with little or no toxicity, containing steroid glycosides and saponins, such as polysponin, diasponin, etc. [5, 7]. The action of these preparations has been tested on animals on a chronic high-cholesterol diet. In 20% of the animals spontaneous atherosclerosis was observed, to which rats are less prone [8, 9, 15]. Atherosclerosis is characterized by successive changes, occurring in stages, in the heart, blood vessels, and other organs. Special attention must be paid to the early stages of atherogenesis with intravasation of serum proteins and lipids into the wall of blood vessels, de-endothelization of their intima, platelet aggregation, accumulation of sulfur-containing glycosaminoglycans, transformation of smooth-muscle cells into foam cells, and fibroelastosis [1, 2, 4, 6, 11]. These changes lead to the formation of lipid streaks and stains, atheromatous plaques, and complicated lesions, reflected in an atherosclerotic index of the I to the IV degree, together with scar changes in the internal organs [1].

The aim of this investigation was a pathomorphological evaluation of the action of furostanol glycosides and saponins of plant origin on experimental atherosclerosis.

EXPERIMENTAL METHOD

The experimental animals were 700 noninbred male albino rats aged 1 year, of which 100 were controls. The other 600 animals were kept for 9 months on a special high-cholesterol

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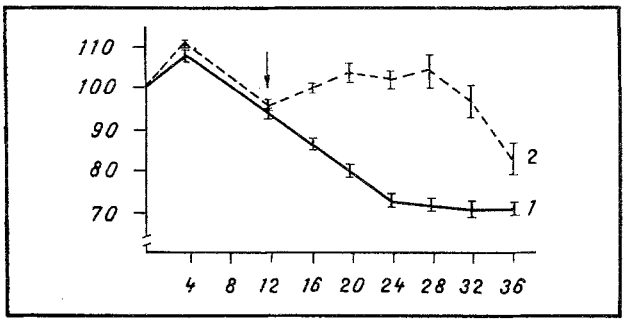

Fig. 1. Time course of body weight of experimental rats treated with saponins. 1) Model; 2) treatment; arrow indicates beginning of treatment. Abscissa, time of observation (in weeks); ordinate, body weight (in %).

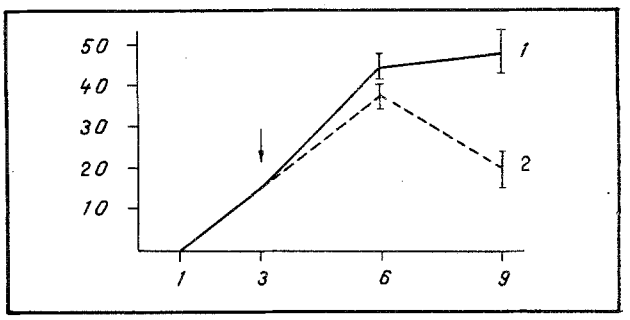

Fig. 2. Effect of saponins on experimental atherosclerosis in rats. Abscissa, time of observation (in months); ordinate, area of lesion of aorta (in %).

diet with 16 additives, including 0.03 mg of mercazolyl (methimazole) and 0.5 mg of ergocalciferol (Wilgram, 1958, 1959, in Bazaz'yan's modifcation), inducing progressive atherosclerosis [3]. In the early stages, daily for 5 months the animals of four groups were treated with plant saponins in doses of 5 and 50 mg/kg. Depending on which preparations were used, the animals were divided into several groups. The data given below are averaged and correlated among groups: control, experimental (9 months on diet), and treated (5 months). Levels of total lipids, cholesterol, and triglycerides were determined in the animals' serum. The ECG was recorded in three standard derivations. In this report particular attention is paid to pathomorphological and morphometric criteria, with determination of body weight and weight of organs with an accuracy of four significant figures; to the direct planimetric parameters of the severity of atherosclerosis and the time course of its morphology in the aorta and coronary arteries (postmortem examination, fixation in isopropyl alcohol, and staining with Sudan) to histopathological and histochemical criteria of the changes in the aorta (hematoxylin-eosin, fuchselin-picrofuchsine, Masson's trichrome, neutral oil red, Sudan black B, 0.1% toluidine blue solution at different pH values (2.3, 7.4, and 11.2; PAS reaction) on microtome section 5-7 μ thick under the light microscope (NU-2, East Germany).

EXPERIMENTAL RESULTS

The body weight of the healthy control rats after 9 months on a normal diet was increased by 110 ± 10 g. The initial serum levels were as follows: total lipids 8.3 ± 1.4 g/liter, cholesterol 1.52 ± 0.22 mmoles/liter, and triglycerides 0.6 ± 0.12 mmole/liter. No abnormalities were found on the ECG in three standard derivations. In animals kept on a diet the body weight increased to 110 ± 2 g, not after 9 months, but after only 1 month, and later it gradually decreased (Fig. 1). The serum biochemical tests also were changed. By the 9th month the total lipid level had increased to 34.7 ± 2.3 g/liter, cholesterol to 5.77 ± 0.33 mmole/liter, and triglycerides to 1.69 ± 0.16 mmole/liter, i.e., by 4 times compared with initially. Meanwhile, the ECG in standard leads I, II, and III, against the background of a general reduction of voltage, especially of the P wave, and a fall in the heart rate, showed lengthening of the PQ and T intervals, i.e., signs of an atrioventricular conduction disturbance with diffuse and microfocal myocardial ischemia, accompanied by

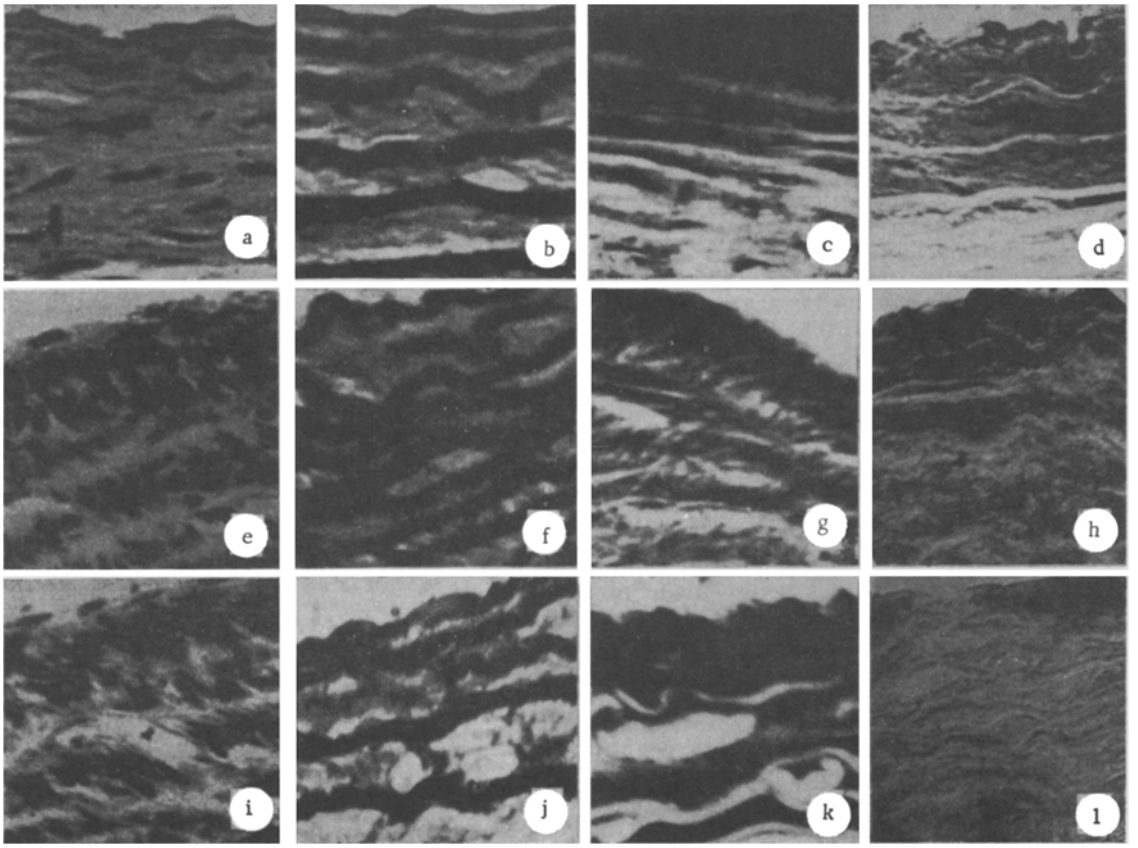

Fig. 3. Morphogenesis of experimental atherosclerosis of aorta in rats under treatment by saponins. Hematoxylin-eosin (a, e, i), fuchselin and picrofuchsine (b, f, j), PAS reaction (c, g, k), and neutral oil red (d, h, l). Magnification 788. a-d) Control animals; a) normal relationship between endothelium of intima and leiomyocytes of media of rat aorta, b) structuring of elastic membranes and picrinophilic zones of aorta, c) distribution of glycosaminoglycans in intima and media of aorta, d) lipids in endothelium; e-h) experimental atherosclerosis of rat aorta: e) de-endothelization of intima with proliferation of leiomyocytes in it and media, f) fibroelastosis, elastolysis, and elastorhexis; g) redistribution of glycosaminoglycans in plaque, h) lipoidosis; i-l) action of saponins on atherosclerosis of rat aorta; l) re-endothelization of plaque, j) reduction of fibroelastosis, k) redeposition of glycosaminoglycans, l) delipidization of intima and media.

hypertrophy of the left ventricle. Morphometric coefficients of relative weight of the animals' internal organs were increased: the heart from 0.50 ± 0.02 to 0.67 ± 0.01 (the index of the left ventricle was increased from 0.50 ± 0.01 to 0.78 ± 0.02), the liver from 1.63 ± 0.23 to 4.14 ± 0.11, the kidneys from 0.29 ± 0.03 to 0.37 ± 0.01, and the lungs from 0.32 ± 0.01 to 0.64 ± 0.02, which can be explained by venous stasis in these organs. The coefficient of the thyroid gland, on the other hand, was reduced from 0.072 ± 0.003 to 0.047 ± 0.006, which confirmed its hypofunction as a result of the methimazole. The change in the weight coefficients of the internal organs were significant ($p < 0.05$). Even more convincing changes were found in the aorta and the coronary arteries. No changes were observed in these arteries in the control rats, even after 9-12 months of observation. Direct planimetry of the intima of vessels removed at autopsy from rats kept on the diet, fixed in isopropylalcohol, and stained with Sudan black B, revealed several degrees of atherosclerosis (stages I-IV), which after 3 months of the diet affected 15% of the total area of the intima of the aorta and coronary arteries, rising to 45% after 6 months and 48% after 9 months, Complicated lesions (thrombosis,calcification,ulceration and hemorrhage into atheromas) were found less frequently, lipoidos, plaques, and atheromatous lesions more frequently. Taking the atherosclerotic index and its component elements into account [1] and, depending on the duration of the diet, several different forms were observed (1 — lipoidosis, 2 —

plaques, 3 — atheroma, 4 — complicated lesions). The following were the predominant forms: after 3 months on the diet: 15 (10 + 3 + 1.5 + 0.5), after 6 months 45 (19 + 12 + 11 + 3), and after 9 months 48 (18 + 9 + 13 + 5). Taking account of average values and their deviations of the intervals and parameters, and significant differences in the fully representative groups, the index of accuracy did not exceed 3% ($p < 0.03$) and the coefficient of correlation (r) did not fall below 0.7 for any of the structures compared (Fig. 2). Atherosclerotic changes predominated in the abdominal and thoracic divisions of the aorta, and in the circumflex and interventricular coronary arteries, but stenosis of their lumen did not exceed 20%. Myocardial infarction was not observed, but there were many tiny perivascular scars in the hypertrophied left ventricle. Such scars also were found in the renal cortex, and in the renal pelvis amorphous, yellowish concrements with cholesterol crystals were sometimes found. Congestive phenomena predominated in the lobules of the liver, while in the thyroid gland there was atrophy of the glandular epithelium of the follicles and sclerosis of the stroma, confirming hypofunction of the gland. Details of the morphogenesis of atherosclerosis in the rats were obtained in serial sections through the aorta. In control animals both spontaneous fibrosis in the aorta and sclerotic changes were absent. With lengthening of the period on the diet and progression of atherosclerosis in the aorta focal destruction of the intima was observed with areas of de-endothelization and disappearance of the thin layer of glycosaminoglycans from the inner surface, intravasation of blood serum into and beyond the vessel, and accumulation of lipid granules and drops in the endothelial and smooth-muscle cells of the intima and media. In the subendothelial layer and intercellular spaces glycosaminoglycans, chiefly with acid radicals, were deposited. Focal elastorrhexis, elastolysis, and fibroelastosis were observed in the elastic membranes, and with progression of the disease, these changes progressed to collagenization and hyalinosis. Fat-loaded smooth-muscle cells were transformed into "foam" cells (Fig. 3, a-h). In these areas plaques and atheromatous lesions were formed by conversion from lipid stains and fibromuscular foci into fibrous, atheromatous, and hyalinized plaques, i.e., progression of atherosclerosis took place. Atheromatous and scar changes and hyalinosis did not undergo regression under the influence of saponin treatment, and attention was therefore concentrated on changes characteristic of the early stages of atherogenesis.

After daily treatment for 5 months with the plant preparations containing furostanol glycosides and saponin, several changes were observed in the animals. Serum levels of total lipids fell compared with the model from 34.7 ± 2.3 to 25.1 ± 1.8 g/liter, cholesterol from 5.77 ± 0.33 to 4.29 ± 0.09 mmole/liter, and triglycerides from 1.69 ± 0.16 to 0.79 ± 0.5 mmole/liter. If not complete, at least partial normalization of the ECG parameters was observed, with shortening of the PQ and T intervals, an increase of voltage, and enlargement of the P wave, with reduction of the heart rate, i.e., evidence of improvement of atrioventricular conduction and reduction of myocardial ischemia. The morphometric parameters also improved, but without reaching the initial values. The animals' body weight decreased by 72.0 ± 6.0 g. The weight coefficients of the organs decreased: the heart from 0.67 ± 0.01 to 0.58 ± 0.02, the liver from 4.14 ± 0.11 to 3.85 ± 0.21, the kidneys from 0.37 ± 0.01 to 0.31 ± 0.02, and the lungs from 0.64 ± 0.02 to 0.43 ± 0.01. The coefficient of the thyroid gland, on the other hand, rose from 0.047 ± 0.006 to 0.059 ± 0.05 due to restoration of the normal number and size of the follicular epithelial cells and predominance of the macrofollicular type of structure. On planimetric evaluation of the aorta and coronary arteries of the rats after treatment, the atherosclerotic index was reduced by 28% and showed qualitative changes also: 20 (3 + 5 + 10 + 2) compared with the model, which was 48 (18 + 9 + 13 + 5). The index of accuracy $p < 0.03$ and coefficient of correlation $r > 0.7$. Histopathological investigation of the heart revealed a reduction of the number and area of spread of foci of myocytolysis and coagulation necrosis in the myocardium, absorption of thrombi in the coronary arteries, disappearance of lipoidosis, and limitation of the size of the fibromuscular and fibrous plaques and atheromatous lesions. In the hepatic lobules the manifestations of venous stasis were reduced, the vascular bed in the kidneys was restored to normal with disappearance of nephrolithiasis, and the hypervolemia and edema in the lungs, were diminished. However, hypertrophy of the left ventricular myocardium and the scars in it and in the other organs showed no sign of regression. Regression of atherosclerosis in the aorta as the period of treatment lengthened was accompanied by reduction of lipoidosis, plaques, atheromatosis, and complicated lesions (Fig. 2). Histopathological investigation revealed re-endothelization of the intima, reduction of the number of proliferating and fat-infiltrated smooth-muscle cells, of deposited and glycosaminoglycans, and of foci of fibroelastosis (Fig. 3, i-l).

Thus progressive experimental atherosclerosis was induced in 600 noninbred male rats by means of a high-cholesterol diet with additives, against the background of induced thyroid gland hypofunction, and after intensive treatment for 5 months with preparations of plant origin, containing saponins and furostanol glycosides, the atherosclerosis underwent regression. Scars, hyalinosis, and complicated atherometosis did not undergo regression. In the early stages of experimental atherosclerosis treatment was most effective. The results suggest not only a hypolipidemic [5, 7], but also a more complex, direct angioprotective action of these therapeutic agents, restoring normal metabolism in the arterial wall. With this action in mind, and also the absence of any toxico-allergic side effects of these therapeutic preparations, it may be suggested that they have a positive role in the prevention and treatment of uncomplicated atherosclerosis.

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