EFFECT OF INHIBITORS OF LIPOLYTIC ACTIVITY ON DEVELOPMENT OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS IN RABBITS

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UDC 616.13-004.6-092.9-092:577.153.2.025.3

Considerable importance in the pathogenesis of experimental atherosclerosis is attached to the depressed lipolytic activity of the blood serum, the wall of the aorta, and other organs [2, 9, 10, 14]. However, this view is based mainly on the fact that a relatively low lipolytic activity is observed in rabbits in normal conditions and when kept on a high cholesterol diet.

In the present experiments an attempt was made to discover the effect of inhibition of lipolytic activity on the development of lipoidosis of the aorta in rabbits. The inhibitors of lipolytic activity selected for the investigation were quinine dihydrochloride [1, 5, 7, 11-13] and chloroquine.

EXPERIMENTAL METHOD

Experiments were carried out on 16 male chinchilla rabbits weighing 2700-3400 g, receiving cholesterol for 60 days in a daily dose of 0.2 g/kg body weight mixed with finely chopped cabbage. The animals of group 1 (6 rabbits) were controls, the animals of group 2 (5 rabbits) received at the same time 125-250 mg chloroquine daily by mouth, while the animals of group 3 (5 rabbits) were also kept on a high cholesterol diet and received at the same time 10 ml of a 1-2% solution of quinine dihydrochloride daily by intramuscular injection (to produce a quinine depot, in the manner described by A. B. Khavkin [4]). The rabbits of groups 2 and 3 received for 3 days a preliminary course of injections of the corresponding inhibitor. At the beginning of the experiment, after this preliminary course, and also after the 30th and 60th days of the experiment, the lipolytic activity of the blood serum was determined by Barreto's method [6], using milk as substrate (at the rate of 0.2% fat/ml blood serum); the fibrinolytic activity and fibrinogen of the blood were determined by Bidwell's method, the blood heparin by Pieptea's method, and the serum cholesterol by Bloor's method. All the rabbits were kept in identical conditions and on an ordinary diet.

At the end of the experiment the animals were decapitated. The nonspecific esterase activity was studied histochemically by Gomori's modification of the method of Nachlas and Seligman, and the anisotropic lipids were studied in polarized light in sections of the liver, pancreas, and aorta. The segment of the aorta of the rabbits from the mitral valves to the bifurcation was extracted, fixed in 10% neutral formalin, stained with Sudan III, and studied by V. S. Smolenskii's combined planimetric and gravimetric method [3].

EXPERIMENTAL RESULTS

After administration of 250 mg chloroquine by mouth to the rabbit (group 2) daily for 3 days and 10 ml of a 1% solution of quinine dihydrochloride in water and glycerol intramuscularly (group 3) relative inhibition of the lipolytic activity of the blood serum from 0.7±0.2 to 0.2±0.0109 meq/liter and from 0.7±0.1 to 0.5±0.2 meq/liter respectively was observed. At the same time the fibrinolytic activity of the blood rose from 5.5±2.8 to 22.6±6.9% (group 2) and from 6.9-4.3 to 15.9±9.0% (group 3).

In the control rabbits kept for 30 days on a high-cholesterol diet and receiving chloroquine in addition, a further depression of the lipolytic activity was observed to 0.11±0.03 meq/liter (group 1) and 0.12 ±0.08 meq/liter (group 2). After 60 days of the experiment marked inhibition of lipolytic activity was found in all the groups, but especially in the experimental groups: 0.04±0.008 meq/liter (group 1), 0.03±0.008 meq/liter (group 2), and 0.008±0.006 meq/liter (group 3). The difference between the initial and final values

Institute of Therapy, Academy of Medical Sciences of the USSR (Presented by Active Member of the Academy of Medical Sciences of the USSR, A. L. Myasnikov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 7, pp. 27-30, July, 1967. Original article submitted July 23, 1965.

TABLE 1. Level of Cholesteromia in Rabbits (in mg %)

Group of animals	Initial values	After 30 days	After 60 days
1	67±6	595±72	920±121
2	60	408±59	734±141
3	60	510±64	715±38

of the experimental groups ($P < 0.02$) and between the final values of groups 1 and 3 ($P < 0.02$) was statistically significant. The fibrinolytic activity in the animals of group 1 was depressed still further — to 1.9±1.2% ($P < 0.05$), in the rabbits of group 2 it was increased to 1.78 ±5.6% ($P > 0.05$), and in the animals of group 3 it was lowered to 8.0±3.5% ($P > 0.05$). A statistically significant difference was also obtained between the final values of groups 1 and 2 ($P < 0.05$).

The fluctuations in the level of the blood fibrinogen and heparin were irregular and not statistically significant. In the animals of all groups the development of hypercholesteremia was observed, and this increased during the time the rabbits were fed with cholesterol (see Table 1).

A noteworthy feature was the absence of a statistically significant difference between the levels of the hypercholesteremia in the control and experimental groups.

The degree of lipolytic activity in the sections of the liver, pancreas, and aorta demonstrated histochemically was defined as normal (sections of the organs of healthy rabbits were used as controls), increased, moderately decreased, and low, and absence of activity was also noted. In the animals of the control group the lipolytic activity in the liver sections was moderately or considerably decreased; in two rabbits of group 2, it was moderately decreased, while in three animals it could not be determined. In the animals of group 3 the pattern was the same. The lipolytic activity in the pancreas of the animals of groups 1 and 2 was increased or moderately decreased, in the rabbits of group 3 the activity was mainly moderately decreased. In the aorta of the experimental rabbits either a very low lipolytic activity was observed or it could not be determined by the histochemical method.

Investigation of the sections in polarized light revealed no regular abnormalities in the accumulation of anisotropic lipids in the animals of the experimental groups. Among the rabbits receiving cholesterol only, lipoidosis of the aorta was well defined in 5 of the 6 rabbits, in which it occupied on the average 60.9±8.7% of the total area of the aorta; in one rabbit (No. 6) the lipoidosis of the aorta was moderate — 11.0% of the area of the aorta. In the experimental groups lipoidosis of the aorta was much less widespread: 38.5±3.5% (group 2) and 28.2±8.6% (group 3) of the area of the aorta. The difference from the control group was statistically significant ($P < 0.01$)

Consequently, in the animals receiving inhibitors of lipolytic activity (chloroquine or quinine dihydrochloride) during high cholesterol feeding, against the background of depression of the lipolytic activity the lipoidosis of the aorta was less severe — on the average the extent of its distribution was reduced almost by half. This difference was particularly clear when the areas involved with thick plaques were compared in the control and experimental groups: in group 1 — 25.4±10.1%, in group 2 — 5.0±2.2%, and in group 3 — 6.5±1.6%, so that in the experimental groups the thick plaques occupied on the average an area only 20-25% as great as in the control group.

In the present experiment quinine and chloroquine mainly inhibited the lipolytic activity of the blood serum and liver, whereas the pancreatic lipase was resistant, a finding not in agreement with reports in the literature regarding quinine [12]. Besides the quinine previously used, chloroquine (which is less toxic than quinine) may also be used as an inhibitor of lipolytic activity. Attention was drawn to the ability of the inhibitors of lipolytic activity to increase at the same time the fibrinolytic activity in the rabbits.

Finally, the results obtained show that the inhibition of lipolytic activity by quinine dihydrochloride and chloroquine did not give rise to an increase in the severity of lipoidosis of the aorta in experimental cholesterol atherosclerosis, despite the approximately equal degree of hypercholesteremia.

The artificially produced depression of lipolytic activity in the present experiments thus did not produce an increase in lipoidosis of the aorta; this does not confirm the importance of depressed lipolytic activity as a factor responsible for the development of experimental alimentary cholesterol atherosclerosis in rabbits.

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