ROLE OF LIPOLYTIC ENZYMES IN THE DEVELOPMENT OF HYPERLIPIDEMIA INDUCED BY HETEROLOGOUS ALBUMIN IN RABBITS

D. Sanchez Serrano,* N. G. Nikul'cheva, and I. B. Soliternova

UDC 616.153.915-008.61-056.43-092.9-092:616.153.1:577.152.311

KEY WORDS: lipoprotein lipase; hepatic triglyceride lipase; bovine serum albumin; triglycerides; cholesterol.

According to the autoimmune theory of pathogenesis of atherosclerosis suggested by Klimov et al. [1] an important role in the development of this disease is played by immune complexes formed by interaction between very-low-and low-density lipoproteins (VLDL and LDL) with γ-globulin. The formation of immune complexes and their harmful action on tissues have been studied in experiments on animals, especially rabbits, injected with bovine serum albumin (fraction V). These complexes exhibited the strongest noxious action against endothelial cells of capillaries and small arteries. One component of the immune reaction was the development of hyperlipidemia in response to repeated injections of heterologous protein [6]. A combination of these factors led to the more rapid development of atherosclerotic vascular lesions [10, 14]. Meanwhile, after a single injection of heterologous protein, immune complexes were found in the blood only after 2 weeks; the cholesterol (Ch) and triglyceride (TG) levels after this time interval were within normal limits [8, 10, 14]. In the earlier periods, however, no analysis of blood lipids was undertaken.

The chief role in the elimination of TG in the composition of chylomicrons (CM) and of VLDL from blood plasma is played by lipoprotein lipase (LPL), fixed to membranes of endothelial cells of capillaries in peripheral tissues. It has been suggested that another lipolytic enzyme, hepatic triglyceride lipase (HTGL) also plays a part in this process, although its role is less definite [3]. Basal activity of the lipolytic enzymes in human and animal blood plasma is extremely low but it rises sharply after injection of heparin [12], and for that reason postheparin plasma is generally used as the source of LPL and HTGL. Insufficient activity of the latter may be one cause of elevation of the blood lipid level in response to injection of foreign protein. It must be emphasized, however, that until very recently the role of lipolytic enzymes in the development of immune hyperlipidemia has remained unexplained.

In the investigation described below the effect of bovine serum albumin on the blood lipid level and on LPL and HTGL activity was studied after injection of a single dose into rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on male Chinchilla rabbits weighing 2.5-3 kg, kept on an ordinary laboratory diet, and divided into two groups: five rabbits formed the experimental group and another five rabbits the control. The animals were used in the experiments without preliminary starvation.

Albumin (bovine serum albumin, fraction V) was injected intravenously in a dose of 200 mg/kg. Blood was taken 30 min, 1, 3, 6, 24, and 48 h, and 7 and 14 days after the injection. Basal enzyme activity was determined in these samples. In addition, to study the activating effect of heparin on lipolytic enzymes and lipoprotein lipolysis, LPL and HTGL activity was

*Cuba.

Department of Biochemistry, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 1, pp. 42-45, January, 1984. Original article submitted May 31, 1983.

0007-4888/84/9701-0048$08.50

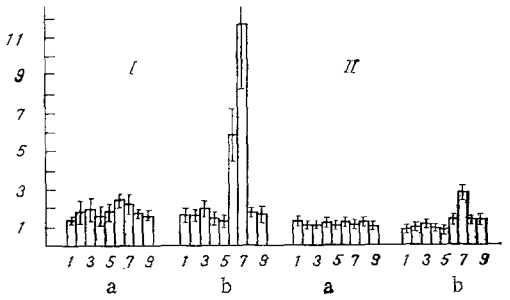

Fig. 1. Plasma triglyceride and cholesterol levels (in mM) after injection of heterologous albumin. a) Control (injection of physiological saline); b) experiment (injection of albumin); I) triglycerides, II) cholesterol. 1) Before injection, 2) 30 min, 3) 60 min, 4) 3 h, 5) 6 h, 6) 24 h, 7) 48 h, 8) 1 week, and 9) 2 weeks after injection.

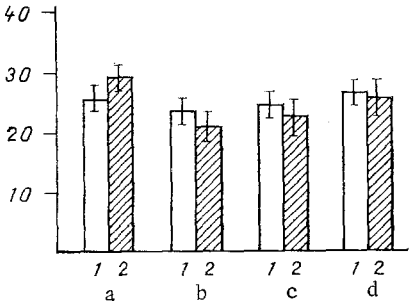

Fig. 2. Activity of postheparin lipoprotein lipase (in mmoles substrate/liter plasma/h) of rabbit blood plasma after injection of heterologous albumin (experiment) and physiological saline (control). 1) Control, 2) experiment. a) 2 weeks before injection of physiological saline and albumin, b) 24 h after injection of those substances, c) 7 days, d) 14 days after injection.

estimated in postheparin blood plasma after injection of albumin. Heparin was injected intravenously in a dose of 200 units/kg body weight 2 weeks before injection of the albumin (initial response) and 24 h and 1 and 2 weeks after injection of albumin solution. In these cases blood was taken before and 10 min after injection of heparin. Solutions of albumin and heparin were injected into the marginal vein of the ear on the side opposite to that from which blood was taken for analysis. The blood was collected in tubes containing sodium citrate as anticoagulant, centrifuged at 800g for 20 min at 4°C, and kept at —30°C until required for analysis.

Animals of the control group received physiological saline in the same volume instead of injections of albumin.

The plasma concentrations of TG and Ch were determined on the AA-2 automatic analyzer (Technicon). LPL activity was measured by the method in [2, 11], HTGL by the method in [4],

TABLE 1. Basal Lipolytic Activity of Rabbit Blood Plasma after Injection of Heterologous Albumin and Physiological Saline (M ± m, n = 5)

Experimental conditions	Unsaturated fatty acids (NEFA), moles/liter plasma/h								
	before injection	time after injection							
		30 min	1 h	3 h	6 h	24 h	48 h	1 week	2 weeks
Heterologous albumin (experiment)	1,12±0,28	1,12±0,36	1,10±0,36	1,25±0,46	2,02±0,41	2,53±0,52*	1,10±0,36	0,78±0,40	0,70±0,25
Physiological saline (control)	1,70±0,54	1,20±0,16	1,40±0,50	1,00±0,07	1,40±0,60	1,70±0,40	1,20±0,40	1,40±0,35	1,90±0,38

Legend. *P < 0.05 compared with initial activity.

TABLE 2. Effect of Heparin on Plasma TG Level in Rabbits after Injection of Heterologous Albumin (M ± m, n = 5)

Experimental conditions	Triglycerides, mmoles/liter plasma/h		% of hydrolyzed triglycerides
	before injection of heparin	after injection of heparin	
2 weeks before injection of heterologous albumin	1,5±0,2	0,90±0,06	40
24 h after injection of albumin	5,8±1,3	4,2±1,2	27,6
7 days after injection	1,7±0,2	1,3±0,2	23,5
14 days after injection	1,6±0,4	1,1±0,5	31,3

but in these experiments intralipid (Vitrum, Sweden) was used as the substrate. The quantity of TG hydrolyzed under the influence of heparin, expressed as a percentage of the TG level before injection of heparin, was calculated in all animals.

The presence of immune complexes in the blood plasma was analyzed by Ouchterlony's double immunodiffusion test.

EXPERIMENTAL RESULTS

A single injection of albumin led to the development of distinct hyperlipidemia in the rabbits, as is shown by the data given in Fig. 1. After 24 h the mean TG level was 262% higher than initially, after 48 h their concentration was increased even more (by 625%), but after 1 week it returned to its initial value. The blood Ch level also was increased at these times, but by a lesser degree than TG (by 80 and 270% respectively). In rabbits of the control group, receiving physiological saline instead of albumin, only a very small increase in the TG concentration was observed within the limits of the usual variations; their Ch level was virtually unchanged throughout the period of observation (Fig. 1).

Simultaneously with the development of hypertriglyceridemia in the experimental animals, a fall was observed in their postheparin LPL activity measured in experiments *in vitro*. It was particularly clearly visible 24 h after injection of albumin (P < 0.05); after 7 days the LPL activity also was below its initial value, although the difference in this case was not statistically significant (Fig. 2).

The level of basal lipolytic activity of the blood plasma (without injection of heparin) showed a less distinct change in response to injection of albumin. It will be clear from Table 1 that the lipolytic activity of the experimental animals began to rise 6 h after injection of heterologous protein and was statistically significantly higher than initially after 24 h; after 48 h it had fallen, and later it was indistinguishable from the initial values. In rabbits of the control group no significant changes were observed throughout the period of observation in the level of basal activity of the plasma lipolytic enzymes.

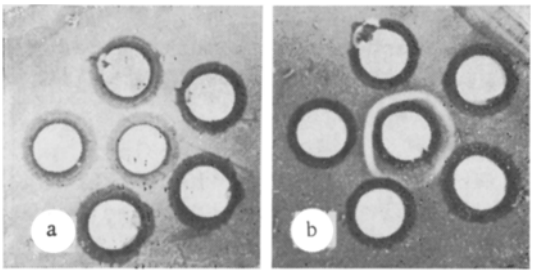

Fig. 3. Formation of immunoglobulins against albumin in rabbits at different times of immunization: a) 1 week, b) 2 weeks after injection of albumin. Central well contains antiserum against bovine albumin, peripheral wells contain solution of albumin in different concentrations (from 0.3 to 0.02 mg/ml).

The degree of lowering of the plasma TG level in response to injection of heparin is an indicator of activation of lipoprotein lipolysis *in vivo*. The hypotriglyceridemic effect of heparin after previous injection of heterologous albumin was found to be weakened to a particularly marked degree 7 days after its injection. For instance, whereas at the first test of heparin the plasma TG level was 40% lower than initially, after 7 days it was only 23.5% lower (Table 2). After 14 days heparin had its usual hypotriglyceridemic effect.

The level of HTGL activity in the postheparin blood plasma showed no significant change in animals of both groups throughout the period of observation.

The results of the double immunodiffusion test (Fig. 3) clearly showed the appearance of a specific precipitation arc after interaction between serum of the experimental animals and bovine serum albumin only when 2 weeks had elapsed after injection of the latter.

The results confirmed those of a previous investigation [8] which showed no change in TG and Ch concentrations 14 days after a single injection of heterologous albumin into rabbits and the appearance of antibodies against albumin at the end of that same period in the rabbits. However, a study of the response of animals to injection of heterologous protein in the earlier stages, in the present investigation, showed marked changes both in the lipid levels and in the activity of plasma lipolytic enzymes. These include, first, the development of definite hypertriglyceridemia, which could be due to some degree to a fall in LPL activity, found after 24 h in the postheparin plasma of the experimental animals. The latter, in turn, could be connected with the harmful action of albumin on capillary endothelial cells [6], on whose plasma membrane LPL is located. As a result there was an increase in spontaneous release of the enzyme into the blood stream, and ultimately this led to a decrease in the capillary LPL pool. This view is supported by data showing an increase in basal lipolytic activity of the blood plasma of rabbits in the early stages (after 6 and 24 h) after injection of albumin. This type of action of heterologous protein could also be connected with the acceptor role of albumin relative to NEFA released in the course of lipoprotein lipolysis, for these substances are inhibitors of LPL [5, 13].

A definite role in the disturbances of TG metabolism induced by heterologous albumin and revealed in these experiments may also be played by secretion of modified lipoprotein particles, which are a worse substrate for LPL than intact CM and VLDL, by the intestine and liver. Judging by the decrease in the content of hydrolyzed TG under the influence of heparin, their accumulation in the blood stream is observed 24 h after injection of heterologous albumin, and is particularly marked 7 days after the injection. However, despite the decrease in the intensity of lipoprotein lipolysis, hypertriglyceridemia was not observed after 7 days, probably because of ingestion of these modified particles by cells of the reticuloendothelial system by nonspecific endocytosis [7]. These results cannot be explained by the presence of antibodies against albumin, for according to data in the literature and our own observations, such antibodies do not appear in the blood plasma until 14 days after injection. Consequently, the causes of development of the hypertriglyceridemia are probably not immunological.

The raised plasma Ch level in the experimental animals was due in all probability to an increase in the content of VLDL, an essential component of which is Ch.

In animals of the control group an increase in the percentage of PG hydrolyzed in the presence of heparin, which is difficult at present to explain, was observed 14 days after injection of physiological saline. The small rise in the TG level observed in the control rabbits after 24 and 48 h could be the result of repeated blood taking [9].

Absence of changes in the activity of the other lipolytic enzyme (HTGL) after injection of heterologous albumin is noteworthy. One cause could be the low basal and postheparin activity of this enzyme in rabbit blood plasma [2]. It is not impossible that albumin does not affect the level of HTGL activity *in vivo*. The results thus suggest that the development of hyperlipidemia in the early stages after injection of foreign protein into rabbits is connected with a fall in activity of postheparin LPL and a possible change in affinity of triglyceride-rich lipoproteins for the enzyme. After 14 days, i.e., by the time of appearance of the immunologic reaction, the normal parameters of lipid metabolism were restored and all that remains in the animals is immunologic memory for the corresponding antigen, so that it can respond more rapidly and more intensively to reimmunization with heterologous protein followed by elevation of the blood lipid level.

LITERATURE CITED

1. A. N. Klimov, Yu. N. Zubzhitsky, and V. A. Nagornev, Atheroscler. Rev., 4, 119 (1979).
2. I. B. Soliternova and N. G. Nikul'cheva, Vopr. Med. Khim., 25, 204 (1975).
3. G. G. Khechinashvili and N. G. Nikul'cheva, Usp. Biol. Khim., 21, 163 (1980).
4. M. L. Baginsky and W. V. Brown, J. Lipid Res., 4, 423 (1977).
5. G. Bengtsson and T. Olivecrona, Eur. J. Biochem., 106, 557 (1980).
6. C. G. Cochrane and P. Koffer, Adv. Immunol., 16, 185 (1973).
7. R. P. Geyer, Physiol. Rev., 40, 150 (1960).
8. Y. Kertulla, T. H. Weber, and O. Wager, Atherosclerosis, 35, 451 (1980).
9. Y. Kertulla, T. H. Weber, and P. Tanner, Atherosclerosis, 38, 321 (1981).
10. H. V. Lamberson and K. E. Fritz, Arch. Pathol., 98, 9 (1974).
11. S. E. Riley and D. S. Robinson, Biochim. Biophys. Acta, 369, 371 (1974).
12. D. S. Robinson and D. R. Wing, in: Adipose Tissue. Regulation and Metabolic Functions, A. Jeanrenaud and D. Hepp, eds., Stuttgart (1970), p. 41.
13. R. O. Scow and T. Olivecrona, Biochim. Biophys. Acta, 487, 472 (1977).
14. H. Van Winkle and L. Levy, J. Exp. Med., 128, 497 (1968).