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Enzyme Histochemical Expression of Lipoprotein Lipase (LPL) in Renal Blood Vessels in Dogs Fed a High-Calorie Diet

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The enzyme lipoprotein lipase (LPL) is synthesized and secreted by adipocytes, myocytes, mammary gland epithelium etc. and is transported to the endothelium of capillaries. LPL is the main lipolytic enzyme participating in the intravascular metabolism of lipoproteins. In the present study, two groups of dogs were used — experimental (obese) and control (non-obese). Immediately after euthanasia, specimens from the renal blood vessels were obtained from both groups. The enzyme histochemical reaction (according to Gomori, 1952) was performed on fresh cryostat sections and by the Tween method, positive expression of LPL in the wall of renal blood vessels in dogs fed a high-calorie diet, was evidenced. In the three vascular layers in control animals, no LPL expression was observed.

Key words: lipoprotein lipase, obesity, high-calorie diet, dogs.

Introduction

Lipoprotein lipase (LPL) is an enzyme that is synthesized and secreted by adipocytes, myocytes (especially cardiomyocytes), the epithelium of lactating mammary gland and other cells. It is transported to the endothelial surface of blood vessels where it exerts its effect. The function of LPL is to hydrolyze triacylglycerols (TAG) from chylomicrons, very low and intermediate density lipoproteins from the luminal side of capillary endothelium [5, 6].

P e n t i k ä i n e n et al. [6] detected LPL in the endothelial cells of capillaries and arteries. K o j a m a et al. [4] reported about endothelial lipase (EL) that is synthesized and secreted mainly by vascular epithelium as a new member of the lipase gene family. Genetically, endothelial lipase is 44% identical to lipoprotein lipase and 41% identical to hepatic lipase according to H i r a t a et al. [1].

LPL expression is immunohistochemically evidenced in the intima, subendothelium, smooth muscle cells and the adventitia of arterial wall [2, 4, 8]. This

shows that subendothelially secreted LPL is not stored in stromal cells where it exerts its functions. In the ischaemic zone of the myocardium throughout sudden death of geriatric dogs, LPL reduction is observed [11, 12].

Mutations in LDL receptors result in a dominant autosomal disease — family hypercholesterolaemia [13, 14]. Mice with severe LPL deficiency die 24 hours after birth. LPL deficiency is also reported in cats and minks. The genetic lack of LPL causes a significant hyperchylomicronaemia, increased plasma TAG concentrations, acute pancreatitis that could be life-threatening [3]. In men such deficiency is encountered in 3-7% of the population [9].

S u s a n n e et al. [10] show that LPL has either a pro- or anti-atherogenic effect depending on its localization. Arterial LPL has a considerable proatherogenic function as it increases the accumulation of intra- and extracellular lipids into the intima [6]. On the contrary, the capillary endothelial LPL is clearly antiatherogenic via its antidyslipidaemic effect [9]. In dogs with moderate and severe atherosclerotic lesions of the aorta, coronary and renal arteries, a significant immunohistochemical expression of the enzyme in endothelial cells, the media, smooth muscle cells and macrophages has been observed. Positive signals for the enzyme were not detected in normal arteries [8]. J o n a s s o n et al. [2] observed the highest LPL amount in smooth muscle cells of blood vessels in vessels affected by atherosclerotic lesions as well as in unaffected arteries. Therefore the authors assumed that vascular smooth muscle cells were the biggest source of LPL.

The aim of the present study was to follow out the enzyme histochemical expression of LPL in renal blood vessels in dogs, fed a high-calorie diet.

Material and Methods

Two groups of dogs — experimental and control were used. The experimental dogs (n=3) had an initial body weight of 12.8667 ± 1.4305 kg. For 90 days, each dog received a daily ration of 400 g canine dry food "Jambo Dog" (Gallisman S. A., Bulgaria) supplemented with 10g/kg b.w. lard, in order to induce experimental obesity. By the end of the high-calorie diet intake, the dogs weighed 16.5417±1.6734 kg. The control group (n=2) weighed 13.654±3.315 kg. Their daily ration consisted of 310 g canine dry food "Jambo Dog" (Gallisman S. A., Bulgaria).

Immediately after the euthanasia of the dogs from both groups, specimens from renal blood vessels (arteries and veins) were obtained. The enzyme histochemical reaction was performed on fresh cryostat sections for detection of positive expression of lipoprotein lipase in vascular walls according to Gomori (1952). The reaction was based upon the Tween method consisting in the deposition of insoluble calcium soaps at the sites of enzyme activity that are further converted to lead soaps and finally, in lead sulfide precipitates. On ready preparations, these precipitates appeared as clusters of dark-brown granules [7].

Results and Discussion

The performed enzyme histochemical studies showed a strong positive expression of LPL in renal blood vessel wall in dogs fed the high-calorie diet. The reaction was detected as presence of dark brown granules — lead sulfide precipitates.

A positive LPL enzyme histochemical expression was observed in the wall of all renal blood vessels in dogs from the experimental group. Positive LPL reaction was

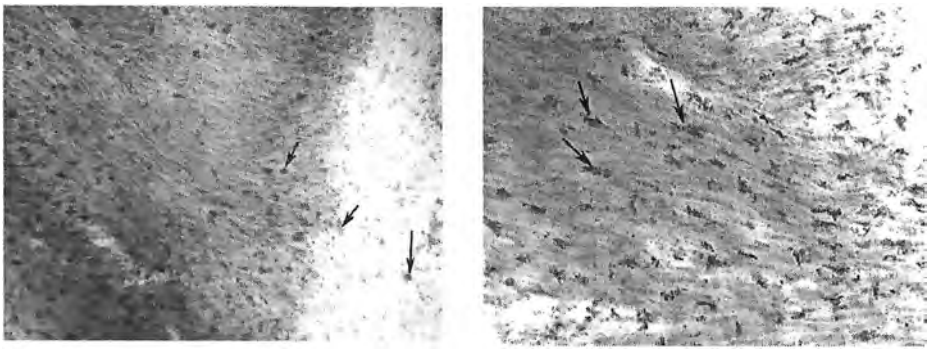

Fig. 1. LPL expression in *Tunica intima* (1a, × 40) and in smooth muscle cells in *Tunica media* (1b, × 100) of dog's renal arteries (arrows)

Fig. 2. LPL expression in the wall of the dog's renal veins (arrows). × 20

detected in the *Tunica intima* of arteries (Fig. 1) and veins (Fig. 3). It was strongly expressed in vascular endothelium.

A similar reaction was observed in connective tissue cells of the subendothelium as well as in sebaceous cells, whose counts were higher as a result of obesity.

In the *Tunica media*, a positive LPL expression was also observed. It was however, the most strongly expressed in smooth muscle cells of arteries (Fig. 2).

Single cells of the adventitia also exhibited a positive LPL reaction. The observations on control dogs revealed no expression of the enzyme in all three blood vessel layers.

As a result of performed studies, it was found out that the high-calorie diet provoked a strong enzyme histochemical expression of LPL in the renal blood vessels of dogs. These data of ours correlate to a certain extent to the results of S a k o et al. [8] for a considerable immunohistochemical LPL expression in the cytoplasm of endothelial cells, smooth muscle cells, of macrophages in the subendothelium, the intima and the media in dogs with lesions of the aorta, the coronary and renal arteries.

We stated that the enzyme histochemical LPL reaction was the strongest in the smooth muscle cells of renal vascular *Tunica media* in the dog. J o n a s s o n et al. [2] had also observed the biggest LPL amount in smooth muscle cells of blood vessels,

both with atherosclerotic lesions and unaffected. This allowed assuming that the greatest source of LPL in blood vessels were vascular smooth muscle cells.

Taking into consideration the proved proatherogenic function of LPL as an enzyme, participating directly or indirectly in the intimal lipoprotein metabolism, augmenting the accumulation of intra- and extracellular lipids into the intima [10], we consider that most probably, the observed enhanced LPL reaction in canine renal blood vessels due to the high-calorie diet, was one of morphological features, perhaps early and prognostic, of atherosclerotic alterations in the wall of these vessels, occurring with obesity.

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