
Vitamin K-dependent carboxylase activity has been detected in human and bovine vessel wall. Studies comparing the carboxylases from liver and vessel wall revealed that the enzyme systems may be regarded as isozymes with widely different substrate specificities. The carboxylated product of vessel wall carboxylase has not yet been identified, but it seems plausible that it will be similar to the GLA-containing proteins which are abundantly present in calcified atherosclerotic plaques (GLA = gamma carboxyglutamic acid, the abnormal amino acid formed by vitamin K-dependent carboxylation). Therefore we have started to characterise the protein constituents of hardened atherosclerotic plaques.

The calcified areas from human aortae were solubilized in EDTA and the proteins extracted were partly purified by batchwise adsorption onto QA-E and elution with high salt. The crude plaque-extract did not contain prothrombin, factor X or protein C. This excludes the possibility that GLA-containing coagulation factors are bound non-specifically from blood. Osteocalcin accounted for 20% of the total amount of protein-bound GLA-residues.

Another GLA-containing protein was purified from the crude plaque-extract by employing high performance liquid chromatography (HPLC). Gel filtration yielded a GLA-rich protein with an apparent Mr of 25 kDa. In vitro both the crude plaque-extract and the purified GLA-containing protein strongly inhibited the precipitation of calcium phosphate and calcium carbonate. A similar effect was not found with human serum albumin nor with a thermally decarboxylated plaque-extract. If also in vivo the GLA-containing proteins produced by vessel wall carboxylase prevent the precipitation of calcium salts remains to be investigated.


Mononuclear phagocytes (M) and vascular cells may participate in the events that lead to the development of atherosclerotic lesions. We have studied the procoagulant activity (PCA) of M and thrombomodulin (TM)-like activity of endothelial cells in 15 rabbits fed an atherogenic diet for 4-5 weeks and in 15 rabbits fed a standard diet. Peripheral blood and spleen M were tested for PCA immediately after isolation (basal PCA) and following in vitro stimulation by bacterial endotoxin, using a one-stage clotting assay. TM-like activity was measured by the rate of (bovine) protein C activation induced by catalytic concentrations of thrombin in the presence of aortic rings (1 cm long) and CaCl2. Blood M expressed negligible basal PCA (< 1 U 1/105 M) both in hyperlipaemic and control rabbits. Endotoxin-injected PCA was not significantly different in the two groups. In contrast, dietary treatment resulted in a significant increase in the basal PCA of spleen M (67.6 ± 13.5 vs 26.5 ± 5.4 U 1/105 M, p < 0.01). Moreover, spleen M from treated animals produced significantly more PCA than controls (p < 0.01) in response to endotoxin. When rabbits were given a single injection of endotoxin, spleen M obtained 60 min after the injection from hyperlipaemic animals expressed 3 times more PCA (p < 0.05, n=6) than did cells from controls. In all instances PCA was identified as tissue factor. TM activity associated with the endothelium was not different in the two groups of animals notwithstanding the presence of fatty streaks on the aortic endothelium of treated rabbits. It is suggested that dietary fats may cause early functional changes in M that lead to increased PCA production both in vivo and in vitro. These data may be relevant to an understanding of the role of M in the pathogenesis of atherosclerosis.


HIGHLY AND VERY HIGHLY DENSITY LIPOPROTEINS ADMINISTRATION INHIBITS PROGRESSION OF EXPERIMENTAL ATHEROSCLEROSIS IN THE RABBIT. J. J. Badimon, L. Badimon and V. Fuster. Division of Cardiology, The Mount Sinai Medical Center, NY, NY.

Epidemiologic studies have shown an inverse relationship between HDL and coronary artery disease. We have previously demonstrated that HDL administration in cholesterol (cho)-fed rabbits, could inhibit the progression of established atherosclerotic lesions. Atherosclerosis was induced by feeding rabbits a 0.5% cho diet for 2 months (160g/day). At that moment, a subpopulation of rabbits (64%) was sacrificed and their aortas showed 50% of aortic atherosclerotic involvement. The remaining animals, kept on the same atherogenic diet, were randomly divided in two identical groups (N=7) : a control and a treated group administered with 50 mg of HDL-cho-VLDL a week for 4 weeks. HDL-cho-VLDL fraction was isolated from normal rabbit plasma by ultracentrifugation at a density range of 1.063-1.25g/ml. The amount of HDL-cho-VLDL administered was determined by its protein content according to Lowry's technique. The 50mgl of HDL-cho-VLDL, measured as protein, contained 1.4mg of total cholesterol, 1.4mg of triglycerides and 0.9mg of phospholipids. At sacrifice, the treated group showed a marked decrease on the extent of aortic by fatty streaks (20 ± 6% X 1SE) as compared to (36 ± 6) in the control group (p < 0.05). Similar results were obtained in aortic wall lipid accumulation (See table, results expressed as X ±SEM; mg/gr dry aorta.)

In conclusion, administration of HDL-cho-VLDL induced a marked inhibition on the progression of atherosclerosis in cholesterol-fed rabbits.

Heparin inhibits smooth muscle cells and endothelial cells. It inhibits the proliferation of the smooth muscle cells and modulates the growth of endothelial cells. Fibroblasts which represent another cell type belonging to the vascular wall could also have their growth modified by heparin. We have at first, demonstrated that I) unfraccionated heparin inhibits the proliferation of the smooth muscle cells and II) unfraccionated heparin, but at a less extent than cold unfraccionated heparin (90%). As it has been reported that HDL-VHDL, measured as protein, contained 1.4mg of total cholesterol, 1.4mg of triglycerides and 0.9mg of phospholipids. Treatment of aortic atherosclerotic involvement. The remaining animals, kept on the same atherogenic diet, were randomly divided in two identical groups (N=7) : a control and a treated group administered with 50 mg of HDL-cho-VLDL a week for 4 weeks. HDL-cho-VLDL fraction was isolated from normal rabbit plasma by ultracentrifugation at a density range of 1.063-1.25g/ml. The amount of HDL-cho-VLDL administered was determined by its protein content according to Lowry's technique. The 50mgl of HDL-cho-VLDL, measured as protein, contained 1.4mg of total cholesterol, 1.4mg of triglycerides and 0.9mg of phospholipids. At sacrifice, the treated group showed a marked decrease on the extent of aortic by fatty streaks (20 ± 6% X 1SE) as compared to (36 ± 6) in the control group (p < 0.05). Similar results were obtained in aortic wall lipid accumulation (See table, results expressed as X ±SEM; mg/gr dry aorta.)

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