

GLA-CONTAINING PROTEINS FROM CALCIFIED HUMAN ATHEROSCLEROTIC PLAQUES. L.J.M. van Haarlem, H.C. Hemker, B.A.M. Soute and C. Vermeer. Department of Biochemistry, University of Limburg, Maastricht, The Netherlands

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 isoenzymes 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 found amongst the Gla-containing proteins which are abundantly present in calcified atherosclerotic plaques (Gla= gammacarboxyglutamic acid, the abnormal amino acid formed by vitamin K-dependent carboxylase). Therefore we have started to characterize 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 QAE 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 kD. 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.

HIGH AND VERY HIGH 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 in vivo administration of HDL-VHDL inhibits the development of atherosclerosis in cholesterol (cho)-fed rabbits. In the present study we have analyzed whether high levels of the physiological cholesterol acceptor, homologous HDL-VHDL, could inhibit the progression of established atherosclerotic lesions. Atherosclerosis was induced by feeding rabbits a 0.5% cho-rich diet for 2 months (140g/day). At that moment, a subgroup of animal (N=4) was sacrificed and their aortas showed 30 ± 8% 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-VHDL a week for 4 weeks. HDL-VHDL fraction was isolated from normal rabbit plasma by ultracentrifugation at a density range of 1.063-1.25g/ml. The amount of HDL-VHDL administered was determined by its protein content according to Lowry's technique. The 50mg of HDL-VHDL, measured as protein, contained 1.4mg of total cholesterol, 1.43mg of triglycerides and 0.6mg 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 ± 1SEM; mg/gr dry aorta.)

	Total Cho	Free Cho	Cho-Esters	TGL	PL
Treated	8.1±1.7	1.7 ±0.4	6.3±1.8	5.5±0.7	3.9±0.4
Control	18.5±1.8	3.04±0.5	15.2±1.4	6.2±1.8	5.5±0.6
P	<0.005	0.02	0.05	NS	0.05

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

MONOCYTE-MACROPHAGE PROCOAGULANT ACTIVITY AND ENDOTHELIAL THROMBOMODULIN IN RABBITS FED AN ATHEROGENIC DIET. P. Montemurro, N. Pasquetto, E. Curci, R. Triggiani, M. Colucci and N. Semeraro. Istituto di Patologia Generale, Università di Bari, Bari, Italy.

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 CaCl<sub>2</sub>. Blood M expressed negligible basal PCA (<1 U/10<sup>5</sup> M) both in hyperlipaemic and control rabbits. Endotoxin-induced 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/10<sup>5</sup> 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 harvested 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.

HEPARIN STIMULATES FIBROBLAST GROWTH INDUCED BY PDGF. E. Dupuy, P.S. Rohrligh, G. Tobelem. INSERM U 150 and CNRS UA 334, Hôpital Lariboisière, Paris, France.

Heparin binds to 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 <sup>125</sup>I unfractionated heparin binds to cultured human skin fibroblasts with a Kd of 1.16 10<sup>-8</sup> M. A low molecular weight heparin fraction (PK 10169) competed (50 %) with <sup>125</sup>I unfractionated heparin, but at a less extent than cold unfractionated heparin (90 %). As it has been reported with endothelial and smooth muscle cells, about 30 % of the bound unfractionated heparin was internalized by the fibroblasts. Heparin alone at the concentration ranges from 0 to 10<sup>-3</sup> M has no effect on fibroblast proliferation measured by the <sup>3</sup>H thymidine uptake. When the cell proliferation was induced by pure PDGF, heparin potentiated markedly the fibroblast growth. The effect started at 10<sup>-6</sup> M heparin and reached a plateau from 10<sup>-8</sup> M to 10<sup>-3</sup> M. Similar stimulation was observed when the growth was induced by FGF or EGF. Low molecular weight heparin enhanced the fibroblast proliferation induced by PDGF but at a less extent than unfractionated heparin, chondroitin sulfate has no effect. When added during the cell culture growth with human serum (5 %), unfractionated heparin increased by 48 %, the cell proliferation as measured by cell counting at the 6th day of the culture. PDGF did not modify the heparin binding on fibroblast cultures either at 4°C or 37°C and did not alter the process of heparin internalization. PDGF binding to the cultured fibroblast (Kd 10.1 ± 3.4 10<sup>-10</sup> M) was not modified by the presence of heparin when studied at 4°C.

In conclusion : i) cultured human fibroblasts bind and internalize heparin, ii) heparin and heparin fraction stimulate the fibroblast growth induced by PDGF, iii) since the binding of PDGF is not modified by bound heparin, the mechanism of stimulation remains unknown.