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Abstracts

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PLASMA LIPOPROTEIN(a) IS AN INDEPENDENT RISK FACTOR FOR CAROTID ATHEROSCLEROSIS ESPECIALLY IN THE PRESENCE OF HIGH LEVELS LDL-CHOLESTEROL LEVELS.

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Clinical studies have suggested that elevated plasma Lp(a) levels may be considered as an independent risk factor for vascular disease especially in the presence of elevated concentrations of LDL-cholesterol, which are themselves atherogenic. In order to evaluate whether high levels of low density lipoprotein cholesterol (LDL-C) may contribute to the atherogenic effect of lipoprotein(a) [Lp(a)], the association between elevated Lp(a) levels and thickening of the intima plus media in the common carotid artery (CC-IMT) in patients with different degrees of hypercholesterolemia was investigated. 100 type II hypercholesterolemic patients and 25 normolipidemic subjects were enrolled in the study. Plasma lipid and lipoprotein levels were determined enzymatically, Lp(a) levels by ELISA. Patients were arbitrarily divided into two groups according to plasma Lp(a) concentration (Lp(a) < 30 or ≥ 30 mg/dl). For each patient mean CC-IMT was determined by B-mode ultrasound using a Biosound 2000 II; in 60 patients and in the controls, the maximal IMT (Max-IMT) in the whole carotid tree was also determined. CC-IMT values were higher in hypercholesterolemic patients with plasma Lp(a) levels > 30 mg/dl than in those with lower levels ($p<0.002$). The CC-IMT and the Max-IMT were directly and independently correlated with plasma levels of Lp(a) ($r= 0.33$ and $r=0.25$ respectively; both $p<0.05$). The effect of LDL-C concentrations on the relationship between IMT and Lp(a) was investigated by dividing the patients into quartiles of plasma LDL-C. After stratification CC-IMT significantly correlated with plasma Lp(a) levels in the patients with severe hypercholesterolemia (LDL-C > 5.2 mmol/L) but not in patients in the lowest quartile of LDL-C, i.e. those with moderate hypercholesterolemia. No correlation between CC-IMT and Lp(a) was found in normolipidemic subjects. Thus elevated plasma levels of Lp(a) can be considered as an additional, independent factor associated with the thickening of common carotid arteries in patients with severe but not in those with moderate hypercholesterolemia or in normocholesterolemic subjects.

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INDUCTION OF PAI-1 BY VLDL IN HepG2 CELLS: INVOLVEMENT OF SIGNALLING PATHWAYS

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We have previously shown that very low density lipoproteins (VLDL) enhance the biosynthesis of plasminogen activator inhibitor type 1 (PAI-1) in HepG2 cells. In this study subconfluent HepG2 cells were incubated for 16 h with 100 μg/ml VLDL in the presence of different inhibitors of signalling pathways. Mepacrine (15μM), a phospholipase inhibitor, completely prevented the enhancing effect of VLDL on PAI-1 secretion. Moreover an increase release of ^{3}H arachidonic acid (45% over basal) was observed in cell preincubated for 16h with labelled arachidonic acid and then challenged with VLDL. Protein kinase C involvement was investigated using a specific inhibitor (H7, 50μM) or by enzyme downregulation by cell pretreatment with PMA (100nM). In these conditions VLDL induced PAI-1 biosynthesis was reduced by 80% and 40%, respectively. The role of calcium was investigated by the use of specific inhibitors added to cell cultures before VLDL. TMB8 (20 μM), which prevents Ca^{2+} release from intracellular stores, reduced PAI-1 secretion by 30%, whereas EGTA (1mM) plus thapsigargin (1-2μM), which induce Ca^{2+} depletion from internal membrane stores, inhibited it by 50%. In contrast, removal of calcium from the cell culture medium with EGTA (1mM), or blocking ions influx with Nifedipine (50μM) did not not prevent PAI-1 induction by VLDL. VLDL induced also tyrosine phosphorilation of proteins with an apparent molecular weight of 80, 70, 44, and 40 KDa. Overall the data indicate that several secondary messenger generating pathways are involved in the induction of PAI-1 biosynthesis by VLDL.

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INDUCTION OF PAI- 1 BY TRIGLYCERIDES AND LINOLEIC ACID IN HepG2 CELLS.

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We have investigated whether intracellular lipid accumulation affects the secretion of plasminogen activator inhibitor type 1 (PAI-1) in HepG2 cells. An apolipoprotein free triacylglycerol (TG) preparation (TGRP, 1-6 mg/ml) obtained from Intralipid®, markedly increased the secretion of PAI-1 by $HepG_2$ after 16 h incubation, and intracellular TG levels. Concomitantly, cellular levels of 18:2 n-6 (linoleic acid, LA) and 18:3 n-3 were markedly raised, reflecting the fatty acid (FA) composition of the supplemented TGRP. To investigate whether the FA components of supplemented lipids were involved in enhancing PAI-1 secretion, cells were incubated with LA or oleic acid (OA), complexed with albumin (LA-BSA or OA-BSA, respectively). LA-BSA concentration-dependently (1-35 μmol/L) enhanced the secretion of PAI-1 into the medium, whereas OA-BSA marginally affected this process. By supplementing cells with increasing (0.5-35 μmol/L) LA, labelled with the ^{14}C isotope, we observed a concentration-dependent incorporation of the FA in cell lipids, with major increments in the TG, diacylglycerol and free FA fractions. Thus, polyunsaturated FA, specifically LA, supplemented to cells, result in their intracellular accumulation, which appear to induce PAI-1 antigen secretion.

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MODIFICATION OF LIPID METABOLISM AND EXPRESSION OF RELATED GENES IN HEMATOLOGIC NEOPLASMS

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Previous studies have shown that hematologic neoplasms are characterized by a reduction of HDL-C levels. In those studies HDL-C was negatively associated with the clinical stage of the disease and clinical remission was accompanied by an increase of HDL-C suggesting a correlation between low HDL-C and intensity of growth rate of tumoral cells. We have suggested that altered HDL pattern in the plasma compartment may be secondary to the altered cholesterol metabolism in leukemic cells. In the current study, the distribution of lipid content in tumor cells and lipoprotein profiles in the plasma were investigated in 30 patients affected by different types of hematologic neoplasms. Our aim was to evaluate whether changes in lipid content in leukemic cells are associated with changes in lipid distribution in the plasma. In addition in leukemic cells we also examined the expression of some genes related with lipid metabolism such as HMGCoA-reductase, LDL receptor and G6PD. The results have shown that the levels of all serum lipid classes did not vary significantly in leukemic patients compared to healthy subjects. In VLDL+LDL fraction a slight increase in triglycerides levels was observed. The HDL fraction revealed a significant decrease of cholesterol, phospholipids and proteins contents. Moreover, the expression of all studied genes was increased in leukemic cells, while a strong decrease in cholesterol and triglycerides content accompanied by an increase of CE/TC ratio was observed in tumoral cells (0.30 vs 0.20 in normal cells). The observations of a low cholesterol content, associated with the high LDL receptor, HMGCoA reductase and G6PD gene expression in leukemic cells, indicate not only an increased demand but also an immediate utilization of cholesterol in order to provide proper amounts of cholesterol for new membrane synthesis and assembly, being leukemic cells characterized by a rapid cell turnover relatively higher than solid tumors. The increased utilization of cholesterol by leukemic cells may also be responsible for the observed inverse correlation between HDL-C levels and growth rate of leukemic cells.