Original articles

Dyslipidemias and fibrinolysis

Luca Puccetti, Fulvio Bruni, Anna Laura Pasqui, Marcello Pastorelli, Giovanni Bova, Michela Cercignani, Alberto Palazzuoli, Alberto Auteri

Department of Clinical Medicine and Immunological Sciences, Section of Medical Semeiotics, Center for Metabolic Diseases and Atherosclerosis, University of Siena, Siena, Italy

Key words:
Cholesterol; Fibrinolysis; Hypercholesterolemia.

Background. **The relation between fibrinolysis and cardiovascular disease is an open debate. Fibrinolysis is related to endothelial function and presents many molecular links with platelet and coagulation activity. Furthermore, reduced fibrinolysis has been reported in several dysmetabolic conditions.**

Methods. **To detect mechanisms linking dyslipidemias and fibrinolysis we evaluated 75 subjects (42 males, 33 females, 20 hypercholesterolemic, 20 hypertriglyceridemic or 20 with mixed hyperlipoproteinemia, 15 with isolated low HDL-cholesterol). Plasminogen activator inhibitor (PAI)-1, tissue-type plasminogen activator activity and plasmin-antiplasmin complexes (PAP) were determined at baseline and after the venous occlusion test. We also measured D-dimer, lipid pattern, soluble E-selectin, platelet surface P-selectin, prothrombin fragments 1+2 (F1+2), lipoprotein(a), factor VII, von Willebrand factor, plasma insulin, fibrinogen, homocysteine, thrombin activable fibrinolysis inhibitor (TAFI) activity, thrombomodulin, factor XIII, urokinase-type plasminogen activator.**

Results. **Hypertriglyceridemic patients were found to have lower PAP and D-dimer and higher PAI-1 serum levels (baseline and venous occlusion test, $p < 0.001$ and $p < 0.01$) compared to hypercholesterolemic and control subjects ($p < 0.01$, $p < 0.001$). P-selectin, F1+2 and TAFI were significantly increased only in hypercholesterolemic subjects ($p < 0.001$) and associated with reduced PAP and D-dimer, showing a linear relation with LDL-cholesterol levels ($p < 0.01$, $r = -0.62$ and $p < 0.01$, $r = -0.59$). PAI-1 activity was not different with respect to controls (baseline $p = 0.59$, venous occlusion test $p = 0.42$). Serum levels of von Willebrand factor were significantly increased in hypertriglyceridemic/low HDL subjects compared to hypercholesterolemics ($p < 0.01$).**

Conclusions. **Impaired fibrinolysis in subjects with hypertriglyceridemia/low HDL-cholesterol is associated with increased serum levels of PAI-1 whereas enhanced thrombin generation and TAFI hyperactivity are the main findings in hypercholesterolemia. Such data may suggest the opportunity of evaluating several fibrinolytic factors when studied as prognostic factors in diverse dyslipidemias.**

(Ital Heart J 2002; 3 (10): 579-586)

© 2002 CEPI Srl

Received April 26, 2002; revision received August 26, 2002; accepted August 29, 2002.

Address:

Dr. Luca Puccetti

Istituto di Semeiotica Medica
Università degli Studi
Policlinico Le Scotte
Viale Bracci, 2
53100 Siena
E-mail: puccetti@unisi.it

Introduction

Impaired fibrinolytic endogenous activity has been associated either with venous thromboembolism or with the acute thrombotic complications of atherosclerosis[1-3]. Metabolic disorders such as diabetes mellitus or dyslipidemias are very important risk factors for arterial thromboembolic disease and they have been related to fibrinolytic disorders[4,5]. Endothelial dysfunction is considered the main involved mechanism in fibrinolytic impairment as suggested by an increased plasminogen activator inhibitor (PAI)-1 activity in many conditions[6-8]. Among dyslipidemias, hypertriglyceridemia has been associated with raised PAI-1 levels and fibrinolytic impairment[9-11] whereas a direct correlation between hypercholesterolemia and reduced fibrinolysis has not been clearly described[12,13]. In such patients, the activation of the coagulation cascade and enhanced platelet-dependent thrombin generation are the most common features[12,14-16]. A correlation between high-density lipoprotein (HDL) cholesterol levels and impaired fibrinolysis has been previously described both in association with hypertriglyceridemia as well as independently of the latter[7,17]. Procarboxypeptidase U (ProCPU), also termed thrombin activable fibrinolysis inhibitor (TAFI), is a recently described inhibitor of fibrinolysis[18]. It is activated by the thrombin/thrombomodulin complex representing a strong link between blood coagulation and fibrinolysis[19,20]. Moreover, enhanced TAFI activity has been related to the progression of coronary artery disease[21]. Another link between platelets and fibrinolysis is represented by the latent form of PAI-1 stored in α-granules. However, the contribution of platelet-derived PAI-1 to fibrinolytic activity has not been completely determined *in vivo*[22]. Other components of the fibrinolytic system such as factor XIII and urokinase-type

plasminogen activator (u-PA) could play an important role in thrombosis but further data are required to establish a direct link with the progression of cardiovascular disease[23]. The aim of our study was to determine the degree of fibrinolytic, platelet and coagulation activity (thrombin generation) in patients with various dyslipidemias in order to detect a possible relation between the metabolic and coagulation-fibrinolytic functional pattern. In particular, we evaluated the correlation with the endothelial marker soluble E-selectin and with the von Willebrand factor[24-28], with P-selectin as an indicator of platelet activation[25,26], with prothrombin fragments 1 and 2 (F1+2) as markers of thrombin generation[27] and with TAFI as a possible molecular link between coagulation and fibrinolysis[20].

Methods

Patients. Seventy-five dyslipidemic subjects were enrolled in the study after they had all given their written informed consent; 20 hypercholesterolemic (12 males, 8 females, mean age 46.9 ± 7.8 years, body mass index 24.1 ± 0.6 kg/m^2), 20 hypertriglyceridemic (11 males, 9 females, mean age 46.1 ± 6.2 years, body mass index 25.1 ± 0.5 kg/m^2), 20 mixed hyperlipoproteinemic subjects (10 males, 10 females, mean age 48.9 ± 6.2 years, body mass index 24.2 ± 0.5 kg/m^2) and 15 patients with isolated low HDL-cholesterol (9 males, 6 females, mean age 49.6 ± 6.4 years, body mass index 24.8 ± 0.4 kg/m^2) were selected among those referred to our lipid clinic. In no case did the patient's history include previous vascular symptoms, a personal or family history of deep vein thrombosis, pulmonary embolism or a tendency to bleed or previous or current therapy with antiplatelet, anticoagulant or hypolipidemic drugs. None suffered from diabetes mellitus, renal, liver, infectious, thyroid or malignant disease. Women were of reproductive age and were not on oral contraceptives. Twelve subjects (8 males, 4 females) were smokers (< 5 cigarettes/die) and were equally distributed within groups. Twenty healthy subjects matched for sex and age were evaluated as controls. Other inclusion criteria were normal values of fibrinogen, homocysteine and lipoprotein(a) [Lp(a)] that are all independently related to enhanced coagulation or decreased fibrinolysis and thrombosis[28-30].

Blood was drawn using a 19G needle between 8.00 and 9.00 a.m. in resting and fasting conditions without stasis. The sample was immediately stored in a serum vacutainer for lipid profiling including apolipoproteins A1 and B (Apo-A1, Apo-B), in a 1:9 citrate blood vacutainer for the determination of the serum levels of fibrinogen, factor VIIc, F1+2, plasmin-antiplasmin complexes (PAP), D-dimer, plasminogen, α_2-antiplasmin, TAFI, factor XIII, u-PA, P-selectin, Lp(a), in a lithium heparin vacutainer for soluble E-selectin and in a Biopool Stabylite vacutainer (Biopool, Umea, Sweden) for PAI-1 and tissue-type plasminogen activator (t-PA) activity. A venous occlusion test was also performed by applying an arm cuff on to the upper arm and inflating it until the mean of the systolic and diastolic pressures was reached. The arm cuff was then left inflated for 10 min and blood drawn as described above. Platelets were obtained as follows: trisodium citrated blood was centrifuged at 100 rpm for 20 min at 18°C to obtain platelet-rich plasma. This was separated from the top two-thirds of the supernatant to avoid contamination by other cells. The remaining blood was centrifuged at 1500 rpm for 10 min to obtain platelet-poor plasma. Other samples were immediately centrifuged at 2200 rpm for 20 min at room temperature and then stored at -70°C. Total cholesterol, triglycerides and HDL-cholesterol were determined by conventional enzymatic methods (Boehringer-Mannheim, Mannheim, Germany). All subjects had triglyceride serum levels < 400 mg/dl and low-density lipoprotein (LDL) cholesterol was calculated using the method proposed by Friedewald et al.[31]; Apo-A1 and Apo-B were measured by radial immunodiffusion (Bouty, Milan, Italy). Serum levels of plasminogen and α_2-antiplasmin were assessed by chromogenic assay (Berichrom, Dade-Behring, Marburg, Germany). Fibrinogen was evaluated using the method proposed by Clauss[32] (Multifibren, Dade-Behring) and factor VIIc by the coagulation method (Dade-Behring). F1+2 (Enzygnost F1+2, Dade-Behring), PAP (Enzygnost PAP, Dade-Behring), D-dimer (Asserachrom, Stago, Marseille, France), soluble E-selectin (ReD system, Minneapolis, MN, USA), Lp(a) (American Diagnostica, Greenwich, CT, USA), thrombomodulin (American Diagnostica), factor XIIIa (America Diagnostica), u-PA (American Diagnostica) and von Willebrand factor (American Diagnostica) were determined by ELISA on a BioRad model 550 microplate analyzer (Bio-Rad, Hercules, CA, USA). The platelet expression of P-selectin was determined as previously described[33]. Briefly, the cytofluorimetric detection of the surface P-selectin (CD62p) expression was evaluated using CD62p labeled with phycoerythrin (Becton-Dickinson, Franklin Lakes, NJ, USA) and FITC-labeled anti-CD61-monoclonal antibody (MAb) (Becton-Dickinson) as positive control. Aliquots of platelet-rich plasma (200 µl) were further centrifuged at 800 rpm for 5 min and after plasma discharging, fluorescent MAbs (20 µl) were added directly to platelet pellets and incubated for 30 min at room temperature in the dark. The unbound MAb was removed by addition of Tyrode's buffer (137 mM NaCl, 2.8 mM KCl, 1 mM $MgCl_2$, 12 mM $NaHCO_3$ 0.4 mM Na_2HPO_4, 0.35% serum albumin, 10 mM HEPES, 5.5 mM dextrose pH 7.4) and centrifuged at 1500 rpm for 5 min (twice). The sample's fluorescence intensity was analyzed on FACS-SCAN Cytometer at 488 nm (Becton-Dickinson). Vital platelets were gated on the basis of the forward angle and 90° light scatter parameters. The percent of purity was detected using the anti-CD61-MAb.

For every histogram 50 000 platelets were counted to evaluate the percentage of CD62P positive cells. Plasma insulin was analyzed using a radioimmunoassay method (Phasadeph Insulin RIA 100, Pharmacia Diagnostica, Uppsala, Sweden); PAI-1 and t-PA activity were assessed using the chromogenic method (Spectrolyse/Fibrin, Biopool) on a Beckman DU-7 spectrophotometer (Beckman, Fullerton, CA, USA). Homocysteine was measured by high-performance liquid chromatography (HPLC) as described by Araki and Sako[34]. The ProCPU (TAFI) activity was assessed as described by Schatteman et al.[35]. Briefly, 20 µl of plasma were activated with 20 µl of a thrombin-thrombomodulin complex mixture at room temperature for 10 min (final concentration during activation: thrombin 4 nM, thrombomodulin 16 nM). The plasma was diluted 20-fold in 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-etanesulphonic acid), pH 7.4, and the thrombin-thrombomodulin complex was prepared by adding 1 part of 16 nM thrombin, 64 nM thrombomodulin, 20 mM HEPES, 5 mM $CaCl_2$, 0.01% Tween 80, pH 7.4 to 1 part of 80 mM $CaCl_2$ in 20 mM HEPES, pH 7.4. The activation of ProCPU to active carboxypeptidase was stopped by addition of 10 µl of 20 µM H-D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK, Alexis Biochemicals, Laufelfingen, Switzerland). The carboxypeptidase activity was then measured by means of HPLC using the substrates hippuryl-L-arginine and hippuric acid as a standard[35]. O-methylhippuric acid was used as internal standard and 10 µl of distilled water added to 40 µl of substrate was the blank. One unit of enzyme activity was defined as the amount required to hydrolyze 1 µmol of substrate per minute at 37°C. The carboxypeptidase activity was calculated as the mean ratio of the peak heights of hippuric acid and the peak heights of the internal standard of the sample, blank and calibrator (U/l). The HPLC equipment consisted of a BioRad model 1306 pump and ultraviolet analyzer. Human thrombin was obtained from Sigma (St. Louis, MO, USA), rabbit lung thrombomodulin from American Diagnostica, HEPES from Calbiochem (Dormstadt, Germany), hippuryl-L-arginine, hippuric acid and O-methylhippuric acid from Sigma-Aldrich (St. Louis, MO, USA).

Statistical analysis. All values are expressed as mean ± SD; comparisons between groups were performed using the ANOVA and the Bonferroni-Dann tests. The Spearman's correlation coefficient was calculated in order to evaluate the relationship between variables. A p value of < 0.05 was accepted as statistically significant. All calculations were performed using SPSS library (SPSS Inc., Chicago, IL, USA).

Results

The basic metabolic parameters of patients and controls are shown in table I. We observed a significantly reduced fibrinolytic activity in hypertriglyceridemic subjects with respect to controls and hypercholesterolemic patients (all PAP $p < 0.001$, all D-dimer $p < 0.01$) (Table II) both at baseline as well as after venous occlusion test. This was related to a significant increase in PAI-1 activity, soluble E-selectin, von Willebrand factor and a reduced t-PA activity in hypertriglyceridemic subjects with respect to controls and hypercholesterolemics (all $p < 0.001$) (Table II). Serum levels of the TAFI were not significantly different from controls and did not correlate with triglyceridemia ($p = 0.316$, $r = 0.08$). Data from hypercholesterolemic subjects showed a near-significant reduction in the serum levels of D-dimer in comparison with controls ($p = 0.052$) whereas PAP and PAI-1 were not significantly changed ($p = 0.079$ and 0.099 respectively) neither at baseline nor after venous occlusion test (Table II) (Figs. 1 and 2). However, D-dimer levels were related to significantly higher P-selectin expression, F1+2 and TAFI activity with respect to controls and hypertriglyceridemic subjects at baseline ($r = -0.71, -0.68, -0.73, -0.68$, $p < 0.001$) whereas no significant change was detected for PAI-1 and t-PA ($p = 0.59$ and 0.46 respectively)

Table I. Lipid pattern, fasting insulin and homocysteine levels in dyslipidemic patients and controls.

	Hyper TG (n = 20	Hyper TC (n = 20	Hyper TG and hyper TC (n = 20)	Low HDL-cholesterol (n = 15)	Controls (n = 20)
Total cholesterol (mg/dl)	180.2 ± 13.3	254.7 ± 21.6§	239.8 ± 20.4**	169.4 ± 8.7	174.6 ± 14.1
LDL-cholesterol (mg/dl)	84.1 ± 9.9	181.1 ± 12.7§	133.1 ± 9.5*	112.3 ± 7.5	104.5 ± 8.4
HDL-cholesterol (mg/dl)	36.3 ± 4.8**	45.7 ± 5.3	43.1 ± 5.5	32.5 ± 4.6§	49.7 ± 5.2
Triglycerides (mg/dl)	318.9 ± 31.6§	102.7 ± 11.6	304.6 ± 28.5§	103.9 ± 11.2	90.1 ± 11.4
Apolipoprotein-A1 (mg/dl)	102.6 ± 10.9**	131.3 ± 10.5	109.3 ± 11.1**	108.4 ± 10.8**	135.7 ± 11.8
Apolipoprotein-B (mg/dl)	138.8 ± 12.7	219.8 ± 13.5§	194.7 ± 12.6**	137.5 ± 12.2	136.9 ± 13.1
Lipoprotein(a) (mg/dl)	15.7 ± 4.4	16.6 ± 4.2	17.6 ± 5.2	17.4 ± 4.8	14.8 ± 4.3
Fasting plasma insulin (mU/ml)	11.3 ± 1.5	9.7 ± 1.2	10.9 ± 1.4	10.1 ± 1.3	8.9 ± 1.1
Homocysteine (mmol/l)	12.5 ± 2.2	11.6 ± 2.9	11.4 ± 3.1	11.6 ± 2.5	10.8 ± 2.3

Values are expressed as mean ± SD. Hyper TC = hypercholesterolemics; Hyper TG = hypertriglyceridemics. One-way ANOVA and Bonferroni-Dann test: * = $p < 0.05$, ** = $p < 0.01$, § = $p < 0.001$ vs controls.

Table II. Hemostatic and fibrinolytic patterns in dyslipidemic patients and controls.

	Hyper TG (n = 20)	Hyper TC (n = 20)	Hyper TG and hyper TC (n = 20)	Low HDL-cholesterol (n = 15)	Controls (n = 20)
PAP (μg/l)					
Pre-VO	87.6 ± 16.3*	249.2 ± 39.8§	96.3 ± 23.2*	100.9 ± 16.4*	274.3 ± 43.2
Post-VO	92.3 ± 18.4*	328.4 ± 36.5§	105.2 ± 24.4*	115.1 ± 23.7*	461.5 ± 49.7
t-PA activity (IU/l)					
Pre-VO	0.46 ± 0.23*	0.85 ± 0.21§	0.49 ± 0.25*	0.52 ± 0.22*	0.89 ± 0.20
Post-VO	0.92 ± 0.31*	3.44 ± 0.86§	0.98 ± 0.30*	1.02 ± 0.35*	3.79 ± 0.93
PAI-1 activity (U/l)					
Pre-VO	19.9 ± 3.8*	10.1 ± 2.2§	17.6 ± 3.5*	16.7 ± 2.7*	9.4 ± 2.5
Post-VO	28.3 ± 4.6*	11.1 ± 2.3§	24.3 ± 4.2*	21.8 ± 3.4*	9.9 ± 2.7
D-dimer (μg/l)	58.7 ± 6.5*	78.3 ± 7.4§**	60.6 ± 6.3*	61.8 ± 6.8*	93.8 ± 7.9
Plasminogen (%)	122.7 ± 17.6	100.1 ± 11.4	120.6 ± 13.5	114.6 ± 10.8	102.7 ± 12.1
α_2-antiplasmin (%)	112.7 ± 9.2	95.3 ± 9.1	110.6 ± 8.8	103.2 ± 8.6	94.7 ± 8.4
Factor XIIIa (U/l)	94.6 ± 15.8	118.7 ± 17.8	108.3 ± 15.1	93.1 ± 14.7	88.4 ± 12.7
u-PA (U/l)	62.7 ± 10.4	64.4 ± 11.6	63.1 ± 10.5	60.2 ± 10.3	69.8 ± 11.3
TAFI (U/l)	960.6 ± 99.7	1141.6 ± 99.8*§	994.9 ± 99.6	957.4 ± 98.4	952.6 ± 94.2
Thrombomodulin (ng/ml)	4.83 ± 0.98	5.52 ± 0.95	4.97 ± 0.98	4.96 ± 0.93	4.37 ± 0.92
F1+2 (nmol/l)	1.0 ± 0.2	2.2 ± 0.4*§	1.5 ± 0.2	0.9 ± 0.3	0.8 ± 0.2
Factor VII (%)	111.6 ± 8.7*	96.4 ± 9.6	108.8 ± 8.3	93.3 ± 6.8	90.8 ± 10.6
P-selectin (% positive cells)	7.4 ± 1.7	22.3 ± 3.4*§	9.4 ± 1.9	8.3 ± 1.5	6.2 ± 1.1
von Willebrand factor (IU/l)	116.8 ± 16.6*	73.1 ± 10.8§	113.6 ± 17.5*	111.3 ± 14.8*	68.2 ± 6.3
sE-selectin (ng/ml)	77.4 ± 8.2*	49.6 ± 7.8§	68.3 ± 9.5*	66.1 ± 8.1*	45.1 ± 6.9
Fibrinogen (mg/dl)	285.4 ± 26.6	290.3 ± 19.3	294.2 ± 20.8	284.3 ± 19.7	276.6 ± 28.8

Values are expressed as mean ± SD. F1+2 = prothrombin fragments 1+2; PAI = plasminogen activator inhibitor; PAP = plasmin-antiplasmin complexes; sE-selectin = soluble E-selectin; TAFI = thrombin activable fibrinolysis factor; t-PA = tissue-type plasminogen activator; u-PA = urokinase-type plasminogen activator; VO = venous occlusion test. Other abbreviations as in table I. One-way ANOVA and Bonferroni-Dann test: * = $p < 0.01$ vs controls; § = $p < 0.01$ vs patients with hypertriglyceridemia; ** = D-dimer, patients with hypercholesterolemia vs controls ($p = 0.052$).

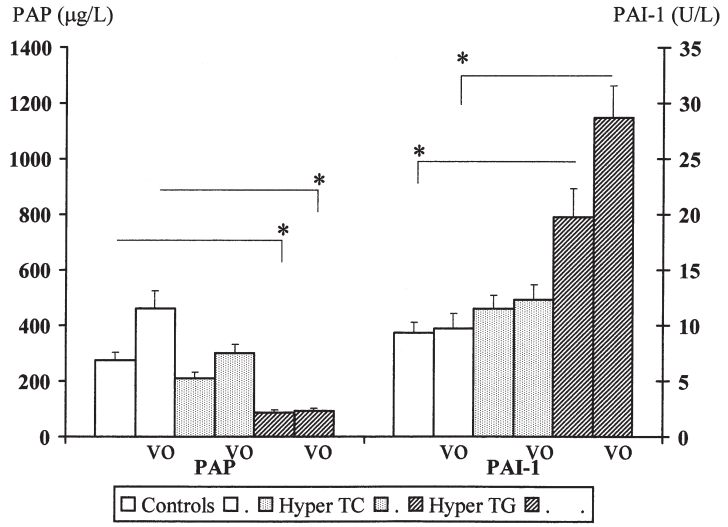

Figure 1. *Plasmin-antiplasmin complexes (PAP) and plasminogen activator inhibitor (PAI)-1 at baseline and after venous occlusion test (VO), in controls (n = 20) and in hypercholesterolemic (Hyper TC) (n = 20) and hypertriglyceridemic (Hyper TG) (n = 20) subjects. All data are expressed as mean ± SD. One-way ANOVA and Bonferroni-Dann test: * = p < 0.01.*

(Fig. 3). Soluble E-selectin and von Willebrand factor were also not significantly increased in comparison with controls ($p = 0.23$ and $p = 0.096$). Subjects with mixed hyperlipidemia showed a significantly reduced PAP, D-dimer and enhanced PAI-1 with respect to controls and hypercholesterolemic subjects ($p < 0.01$ and $p < 0.001$ for both groups) (Table II) both at baseline as well as after venous occlusion test. The TAFI was related to the serum levels of LDL-cholesterol ($r = 0.73$) but was not significantly increased with respect to con-

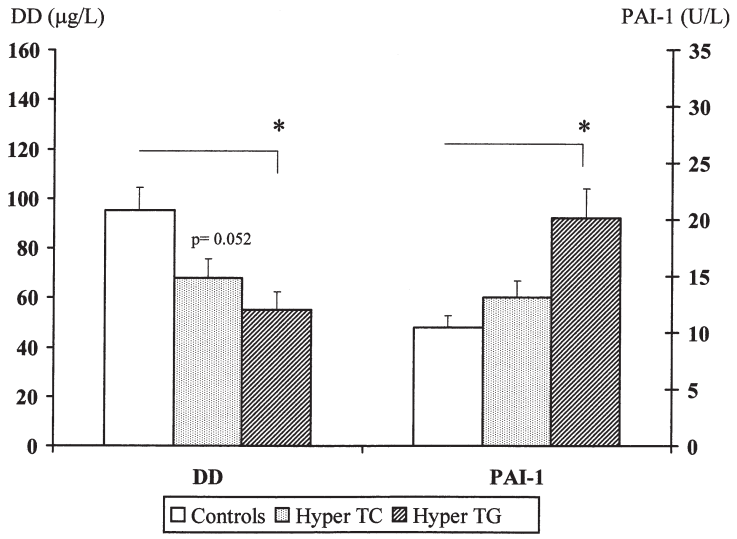

Figure 2. *D-dimer (DD) and plasminogen activator inhibitor (PAI)-1 in controls (n = 20) and in hypercholesterolemic (Hyper TC) (n = 20) and hypertriglyceridemic (Hyper TG) (n = 20) subjects. All data are expressed as mean ± SD. One-way ANOVA and Bonferroni-Dann test: * = p < 0.01.*

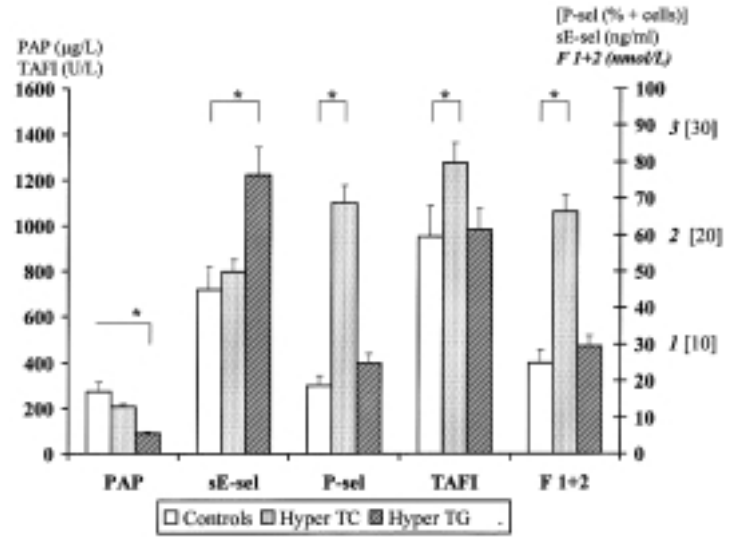

Figure 3. *Plasmin-antiplasmin complexes (PAP), prothrombin fragments 1+2 (F1+2), thrombin activable fibrinolysis inhibitor (TAFI), platelet P-selectin (P-sel), soluble E-selectin (sE-sel) in controls (n = 20) and in hypercholesterolemic (Hyper TC) (n = 20) and hypetriglyceridemic (Hyper TG) (n = 20) subjects. All data are expressed as mean ± SD. One-way ANOVA and Bonferroni-Dann test: * = p < 0.01.*

trols (p = 0.123). Factor VIIc levels were significantly enhanced in hypertriglyceridemic subjects in comparison with controls and hypercholesterolemic patients (p < 0.001 and p < 0.01) showing a direct correlation with the serum levels of triglycerides (r = 0.70) (Table II). Subjects with isolated low HDL-cholesterol showed a fibrinolytic pattern similar to that of hypertriglyceridemic patients at baseline and after venous occlusion test. PAP and D-dimer were significantly reduced (both p < 0.05 at baseline, PAP p < 0.001 after venous occlusion test) and PAI-1, soluble E-selectin and von Willebrand factor enhanced with respect to controls and hypercholesterolemic patients (p < 0.01) (Table II). Serum levels of the TAFI, F1+2, P-selectin and factor VIIc did not significantly differ from those of controls (p = 0.119, p = 0.107, p = 0.208, and p = 0.134 respectively) (Table II). Apo-A1 were significantly lower in hypetriglyceridemic/low HDL-cholesterol subjects (p < 0.01) than in controls and hypercholesterolemic subjects while Apo-B were significantly higher in the hypercholesterolemic group (p < 0.01) (Table I). Fasting insulin levels were slightly higher in the hypertriglyceridemic group than in controls or hypercholesterolemic subjects. However, the difference was not statistically significant (p = 0.101, p = 0.123) (Table I). Thrombomodulin showed no significant difference between controls and hypertriglyceridemic or low HDL-cholesterol subjects (p = 0.126 and p = 0.099). More-

over, it was related to thrombin generation and TAFI activity in hypercholesterolemic subjects (r = 0.57 and 0.61, p < 0.01 and < 0.001) (Table II). No significant difference in the serum levels of u-PA was detected in patients with respect to controls (p = 0.101) and among dyslipidemic subjects (IIa vs IIb, p = 0.139, IIa vs low HDL-cholesterol, p = 0.106, IIb vs low HDL-cholesterol, p = 0.102) (Table II). Serum levels of factor XIII did not significantly differ between controls and patients (p = 0.154) and among dyslipidemic subjects although higher levels were detected in hypercholesterolemics (IIa vs IIb, p = 0.061, IIa vs low HDL-cholesterol, p = 0.083, IIb vs low HDL-cholesterol, p = 0.223) (Table II).

Discussion

A strong link between impaired fibrinolysis and thrombosis has been described both in association with dysmetabolic conditions as well as independently of the latter[36]. Lowering tryglicerides and enhancing HDL-cholesterol by gemfibrozil treatment have been independently associated with an improved fibrinolytic activity[37] and with a reduction in the risk of major cardiovascular events in patients with coronary heart disease[38]. A hypofibrinolyitic state has not been clearly related to hypercholesterolemia and it is suggested that platelet activation and enhanced thrombin generation are the most important mechanisms involved in the development of thrombosis which could be modulated by treatment with statins[39,40]. Moreover, the influence of thrombin generation on fibrinolysis via activation of the TAFI and of factor XIII is well-known[20,41]. Our data confirm the fibrinolytic impairment in patients with hypertriglyceridemia showing a direct correlation between PAI-1 activity and serum levels of triglycerides (r = 0.73) in basal conditions. As stated previously, the post-venous occlusion test raised PAI-1 activity confirms the possible endothelium-dependent mechanism of impaired fibrinolysis[12]. Furthermore, the significant correlation of fibrinolytic activity with the serum levels of soluble E-selectin (PAP r = -0.73, p < 0.01) and of von Willebrand factor (PAP r = -0.68, p < 0.01) supports such a hypothesis. In our population, isolated hypercholesterolemia was associated with a lesser decrease in fibrinolytic activity with respect to other forms of dyslipidemia (p < 0.01). In such patients the fibrinolytic impairment is not related to the PAI-1 activity neither at baseline (r = 0.06, p = 0.59) nor after venous occlusion test (r = 0.09, p = 0.42) suggesting a minor role for endothelial dysfunction in impaired fibrinolysis. Moreover, serum levels of soluble E-selectin and of von Willebrand factor were not significantly different from those of normal subjects (p = 0.37 and p = 0.21) whereas a significant correlation has been found with P-selectin (r = -0.69), F1+2 (r = -0.71) and TAFI (r = -0.70) suggesting a thrombin-mediated fibrinolytic impairment in a multifactorial system comprising thrombomodulin and factor XIIIa[20,41,42]. Such data could indicate a minor role for platelet-derived PAI-1 in a globally hypofibrinolytic state despite the previously described contribution to a local reduction in fibrinolysis[43]. Furthermore, the close relation observed among platelet activity, thrombin generation and high LDL suggests the possibility of a more significant impairment of fibrinolysis in patients with higher LDL levels with respect to our population. Patients with mixed dyslipidemia show a similar pattern to that of hypertriglyceridemic subjects with a significant PAI-1-related hypofibrinolysis (r = -0.75, p < 0.01). In our subjects, isolated low HDL-cholesterol induces a significant reduction in the fibrinolysis related to endothelial dysfunction as shown by the correlation between PAP and PAI-1 activity especially after venous occlusion test (r = -0.73, p < 0.001). Such data could indicate that low HDL-cholesterol is the main determinant of reduced fibrinolysis even in hypertriglyceridemic subjects with low HDL-cholesterol, independently of hypertriglyceridemia. However, in our population HDL-cholesterol is significantly higher (p < 0.05) in patients with mixed dyslipidemia compared to those with hypertriglyceridemia and it is associated with endothelium-related hypofibrinolysis suggesting a direct role of hypertriglyceridemia in such a condition. The relation between isolated low HDL and PAI-1-related hypofibrinolysis strengthen the role of HDL in the maintenance of protective antiatherothrombotic mechanisms. Indeed, a large body of evidence shows that HDL is involved in the regulation of proatherogenic and prothrombotic mechanisms related to endothelial dysfunction especially by counteracting the adverse effects of a relevant atherothrombotic factor such as oxidized LDL[44]. Data about the relation between gemfibrozil treatment, the improvement in fibrinolyis and the progression of coronary artery disease as determined on the basis of angiographic findings are conflicting[45,46]. These studies respectively included patients with combined hyperlipoproteinemia and high levels of total cholesterol[45] and patients with low HDL-cholesterol and moderate hypertriglyceridemia[46]. According to our data, hypercholesterolemia could be a confounding determinant of fibrinolytic activity that could not be modulated by the action of gemfibrozil. Moreover, Lp(a) and homocysteine play a role in blood coagulation and fibrinolysis[29,30] but only in the paper by Bröijersen et al.[45] were the levels of Lp(a) reported while those of homocysteine were not determined neither in this study nor in that by Hamsten et al.[46]. With regard to these observations, the relatively small number of patients included in our study is due to the exclusion of some metabolic conditions related to hypertrigliceridemia and/or hypofibrinolysis such as obesity, insulin resistance[47], hyperhomocysteinemia[29] and high Lp(a)[30] levels. With regard to u-PA, our data were not suggestive of any significant differences and relations in all

groups; however, a study involving the evaluation of its receptor uPAR could provide more information about its involvement in the fibrinolytic status of dyslipidemic subjects.

In conclusion, our data confirm an endothelial-related hypofibrinolysis in patients with hypertriglyceridemia/low HDL-cholesterol. A similar pattern is found in mixed hyperlipidemia with mild hypercholesterolemia and in subjects with isolated low HDL-cholesterol. Compared to controls, patients with type IIa hyperlipoproteinemia show a significant decrease in fibrinolysis related to the higher levels of LDL-cholesterol without showing relevant alterations in endothelial dysfunction. Enhanced platelet activity and thrombin generation are the main findings in such populations and the increased TAFI activity related to thrombin generation may be considered one of the main determinants of the fibrinolytic activity in a complex system including thrombomodulin and factor XIII. Furthermore, such findings may indicate the opportunity of evaluating the different fibrinolytic components as possible prognostic factors in several dyslipidemic conditions.

References

1. Wiman B, Hamsten A. The fibrinolytic enzyme system and its role in the etiology of thromboembolic disease. Semin Thromb Haemost 1990; 16: 207-12.
2. Ridker PM, Hennekens CH, Stampfer MJ, Manson JE, Vaughan DE. Prospective study of endogenous tissue plasminogen activator and risk of stroke. Lancet 1994; 343: 940-3.
3. Thompson SG, Kienast J, Pyke SDM, Haverkate F, van de Loo JCW, for the European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. Hemostatic factors and risk of myocardial infarction or sudden death in patients with angina pectoris. N Engl J Med 1992; 332: 635-41.
4. Gough S, Grant PJ. The fibrinolytic system in diabetes mellitus. Diabet Med 1991; 8: 898-905.
5. ECAT Angina Pectoris Study Group. ECAT angina pectoris study: baseline associations of haemostatic factors with the extent of coronary arteriosclerosis and other coronary risk factors in 3000 patients with angina pectoris undergoing coronary angiography. Eur Heart J 1993; 14: 8-17.
6. Juhan-Vague I, Roul C, Alessi MC, Ardissone JP, Heim M, Vague P. Increased plasminogen activator inhibitor activity in non insulin dependent diabetic patients. Relationship with plasma insulin. Thromb Haemost 1989; 61: 370-3.
7. Cimminiello C, Vigorelli P, Piliego T, et al. Fibrinolytic response in subjects with hypertriglyceridemia and low HDL cholesterol. Biomed Pharmacother 1997; 51: 164-9.
8. Blann AD, Dobrotova M, Kubisz P, McCollum CN. von Willebrand factor, soluble P-selectin, tissue plasminogen activator and plasminogen activator inhibitor in atherosclerosis. Thromb Haemost 1995; 74: 626-30.
9. Metha J, Metha P, Lawson D, Saldeen T. Plasma tissue plasminogen activator inhibitor levels in coronary disease: correlation with age and serum triglyceride concentration. J Am Coll Cardiol 1987; 9: 263-8.
10. Mussoni L, Mannucci L, Sirtori C, et al. Hypertriglyceridemia and regulation of fibrinolytic activity. Arterioscler Thromb 1992; 12: 19-27.
11. Avellone G, Di Garbo V, Cordova R, Raneli G, De Simone R, Bompiani GD. Fibrinolysis in hypertrygliceridaemic subjects in response to venous occlusion. Blood Coagul Fibrinolysis 1993; 4: 429-33.
12. Avellone G, Di Garbo V, Cordova R, et al. Activation of coagulation but normal fibrinolysis in patients with type IIa hyperlipoproteinemia. Thromb Res 1996; 81: 277-82.
13. Hiraga T, Shimada M, Tsukada T, Murase T. Hypertriglyceridemia but not hypercholesterolemia is associated with the alterations of the fibrinolytic system. Horm Metab Res 1996; 28: 603-6.
14. Alessandri C, Basili S, Maurelli M, et al. Relationship between prothrombin activation fragment F1+2 and serum cholesterol. Haemostasis 1996; 26: 214-9.
15. Aoki I, Aoki N, Kawano K, et al. Platelet-dependent thrombin generation in patients with hyperlipidemia. J Am Coll Cardiol 1997; 30: 91-6.
16. Szczeklik A, Musial J, Undas A, et al. Inhibition of thrombin generation by aspirin is blunted in hypercholesterolemia. Arterioscler Thromb Vasc Biol 1996; 16: 948-54.
17. Saku K, Ahmad M, Greenwalt-Glas P, Khashyap ML. Activation of fibrinolysis by apolipoproteins of high density lipoproteins in man. Thromb Res 1985; 39: 1-8.
18. Bajzar L, Manuel R, Nesheim M. Purification and characterization of TAFI, a thrombin-activable fibrinolysis inhibitor. J Biol Chem 1995; 270: 14477-84.
19. Bajzar L, Morser J, Nesheim M. TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascade through the thrombin-thrombomodulin complex. J Biol Chem 1996; 271: 16603-8.
20. Bouma BN, von den Borme PAK, Meijers JCM. Factor XI and protection of the fibrin clot against lysis - a role for the intrinsic pathway of coagulation in fibrinolysis. Thromb Haemost 1998; 80: 24-7.
21. Silveira A, Schatteman K, Goossens F, et al. Plasma procarboxypeptidase U in men with symptomatic coronary artery disease. Thromb Haemost 2000; 84: 364-8.
22. Stiko A, Hervio L, Loskutoff DJ. Plasminogen activator inhibitors. In: Colman RW, Hirsh J, Marder VJ, et al, eds. Hemostasis and thrombosis, basic principles and clinical practice. 4th edition. Philadelphia, PA: Lippincott Williams and Wilkins, 2001: 355-66.
23. Carter AM, Grant PJ. To lyse or not to lyse? Is it genetic? Ital Heart J 2001; 2: 652-5.
24. Gearing AJH, Newman W. Circulating adhesion molecules in disease. Immunol Today 1993; 14: 506-51.
25. Blann AD, Lip GY, Beevers DG, McCollum CN. Soluble P-selectin in atherosclerosis: a comparison with endothelial cell and platelet markers. Thromb Haemost 1997; 77: 1077-80.
26. Stenberg PE, McEver RP, Shuman MA, Jacques YV, Bainton DF. A platelet alpha-granule membrane protein (GMP-140) is expressed on the plasma membrane after activation. J Cell Biol 1985; 101: 880-6.
27. Bauer KA, Rosenberg RD. The pathophysiology of the prethrombotic state in humans: insights gained from studies using markers of hemostatic system activation. Blood 1987; 70: 343-50.
28. Ceriello A, Pirisi M, Giacomello R, et al. Fibrinogen plasma levels as marker of thrombin activation: new insights on the role of fibrinogen as a cardiovascular risk factor. Thromb Haemost 1994; 71: 593-5.
29. Cattaneo M. Hyperhomocysteinemia, atherosclerosis and thrombosis. Thromb Haemost 1999; 81: 165-76.
30. Miles LA, Plow EF. Lp(a): an interloper into the fibrinolytic system? Thromb Haemost 1990; 63: 331-5.

31. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-503.
32. Clauss A. Rapid physiological coagulation method in determination of fibrinogen. Acta Haematol 1957; 17: 237-40.
33. Ferroni P, Speziale G, Ruvolo G, et al. Platelet activation and cytokine production during hypothermic cardiopulmonary bypass. A possible correlation? Thromb Haemost 1998; 80: 58-64.
34. Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr 1987; 422: 43-52.
35. Schatteman KA, Goossens FJ, Scharpé S, Neels HM, Hendriks DF. Assay of procarboxypeptidase U, a novel determinant of the fibrinolytic cascade, in human plasma. Clin Chem 1999; 45: 807-13.
36. Declerck PJ, Juhan-Vague I, Felez J, Wiman B. Pathophysiology of fibrinolysis. J Intern Med 1994; 236: 425-32.
37. Avellone G, Di Garbo V, Cordova R, et al. Improvement of fibrinolysis and plasma lipoprotein levels induced by gemfibrozil in hypertriglyceridemia. Blood Coagul Fibrinolysis 1995; 6: 543-8.
38. Bloomfield Rubins H, Robins JA, Collins D, et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high density lipoprotein cholesterol. N Engl J Med 1999; 341: 410-8.
39. Rosenson RS, Tangney CC. Antiatherothrombotic properties of statins. Implication for cardiovascular event reduction. JAMA 1998; 279: 1643-50.
40. Kearney D, Fitzgerald D. The anti-thrombotic effects of statins. J Am Coll Cardiol 1999; 33: 1305-7.
41. Reed GL, Houng AK. The contribution of activated factor XIII to fibrinolytic resistance in experimental pulmonary embolus. Circulation 1999; 99: 299-304.
42. Mosnier LO, Meijers JCM, Bouma BN. Regulation of fibrinolysis in plasma by TAFI and protein C is dependent on the concentration of thrombomodulin. Thromb Haemost 2001; 85: 5-11.
43. Braaten JV, Handt S, Jerome W, Kirkpatrick J, Lewis JC, Hantgan RR. Regulation of fibrinolysis by platelet-released plasminogen activator inhibitor 1: light scattering and ultrastructural examination of lysis of a model platelet-fibrin thrombus. Blood 1993; 81: 1290-9.
44. Mertens A, Holvoet P. Oxidized LDL and HDL: antagonists in atherothrombosis. FASEB J 2001; 15: 2073-84.
45. Bröijersen A, Eriksson M, Wiman B, Angelin B, Hjemdahl P. Gemfibrozil treatment of combined hyperlipoproteinemia. Arterioscler Thromb Vasc Biol 1996; 16: 511-6.
46. Hamsten A, Syvänne M, Silveira A, et al. Fibrinolytic proteins and progression of coronary artery disease in relation to gemfibrozil therapy. Thromb Haemost 2000; 83: 397-403.
47. Juhan-Vague I, Vague P, Alessi MC, et al. Relationships between plasma insulin triglyceride, body mass index and plasminogen activator inhibitor 1. Diabete Metab 1987; 13: 331-6.