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Research Article

The Impact of a Single Apheretic Procedure on Endothelial Function Assessed by Peripheral Arterial Tonometry and Endothelial Progenitor Cells

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Abbreviations: CPEC: Circulating Endothelial Progenitor Cells; EPC: Endothelial Progenitor Cells; LDL-c: LDL Cholesterol; Lp(a): lipoprotein (a); Ln-RHI: Natural Logarithmic Reactive Hyperaemia Index; PAT: Peripheral Arterial Tonometry; RHI: Reactive Hyperaemia Index

Abstract

Introduction: Endothelial progenitor cells (EPC) are involved in vascular repair and proliferation, contributing to the long-term outcomes of apheretic treatment. Aim of this study was to investigate the relationships between endothelial function, assessed by levels of bone marrow-derived progenitor cells and endothelial response to hyperaemia, and clinical and biohumoral parameters in high vascular risk patients before, immediately after, 24-hours and 72 hours after a single lipid apheresis procedure.

Material and Methods: We evaluated lipid profile, endothelial function and endothelial progenitor cells before (T0), immediately after (T1), 24h after (T2) and 72h after (T3) a lipoprotein apheresis procedure, in 8 consecutive patients [Sex: 62.5% M; Age; 63.29(12), mean, (range) years] with a personal history of acute coronary syndrome, symptomatic peripheral arterial disease and elevated plasma levels of lipoprotein (a) [Lp(a)]. Patients were on regularly weekly or biweekly lipoprotein apheresis, and they were treated with the FDA-approved Heparin-induced Extracorporeal LDL Precipitation (H.E.L.P.) (Plasmat Futura, B.Braun, Melsungen, Germany) technique. PAT values were expressed as the natural logarithm (Ln-RHI, normal values≥0.4) of the reactive hyperaemia index (RHI), which is the parameter automatically calculated by the device.

Results: We found a reduction in the natural logarithm of reactive hyperaemia index (Ln-RHI), assessed immediately after the procedure (0.57±0.21 vs 0.72± 0.29); difference between T2 and T0 was statistically significant (0.43±0.24 vs 0.72±0.29; p=0.006). Reduction in Ln-RHI values was documented in all patients, two subjects showing a Ln-RHI<0.4 at T1, and four at T2. At T3, PAT values were increased significantly (0.91±0.18) in comparison to T1 and T2, showing a median value higher than at T0. Cd34+/Kdr+ and Cd133+/Kdr+ showed a minimum increase in median values at T1, and a higher increase at T2, in comparison to baseline. Differences in Cd34+/133+/Kdr+ values at different times were not statistically significant. A significant reduction in circulating endothelial cells (CEC) count at T2 in comparison to T0 was found (12.00±8.85 vs 23.86±12.39; p=0.024).

Discussion: At 24h and 72h after procedures, we found an improvement in endothelial function, expressed by an increase in PAT values and EPC levels, and by a reduction in CEC.

Introduction

The accumulation of cholesterol in the intima-media of arteries is associated to endothelial dysfunction and pro-inflammatory burden, which constitutes the first step of atherosclerotic progression [1]. Lipid-lowering therapies are the cornerstone of prevention of cardiovascular disease, and, in selected higher-risk patients, lipoprotein-apheresis could be added to standard therapy to optimize secondary prevention [2,3] and improve prognosis.

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Acute and chronic effects of lipoprotein-apheresis were extensively investigated in a recent review [4]. Regarding acute effects, previous studies demonstrated that a single apheretic procedure positively affects endothelial function, assessed as the vasodilatation of both brachial [5] and epicardial coronary arteries [6]; however, Authors investigated only the 24-hours effects after procedures.The long-terms of apheretic therapy are the improvement of lipid profile [7], and the stabilization of atherosclerotic lesions, particularly by the reduction of circulating pro-thrombotic factors [7].

Accordingly with two recent studies [8,9], endothelial progenitor cells are involved in vascular repair and proliferation, contributing to the long-term beneficial effects of apheretic treatment. The reduction of pro-inflammatory and pro-coagulant effects, in addition to the direct reduction of plasma LDL-c levels, could contribute to vascular protection. Aim of this study was to investigate the relationships between endothelial function, assessed by levels of bone marrow-derived progenitor cells and endothelial response to hyperaemia, and clinical and biohumoral parameters, in high vascular risk patients before, immediately after, 24-hours and 72 hours after a single lipid apheresis procedure.

Material and Methods

Study population

The study population consists of 8 consecutive patients [Sex: 62.5% M; Age; 63.29(12), mean, (range) years] with a personal history of acute coronary syndrome, symptomatic peripheral arterial disease and elevated plasma levels of Lp(a), undergoing chronic apheretic treatment for more than one year [2(1-4) years] (according to current American Society for Apheresis (ASFA) guidelines) [10]. Stable CAD was documented by a coronary angiography. Inclusion criteria comprised: 1) Lipoprotein(a) plasma levels >600 mg/L; 2) CAD documented by a coronary angiography; 3) LDL-c levels within targets expected for high risk intervention patients, according to ESC guidelines; 4) lower or no response to maximal hypolipemic pharmacotherapy. Exclusion criteria comprised: 1) autoimmune diseases; 2) cancer or history for cancer; 3) acute or chronic disease affecting liver or kidney function; 4) acute illness at the visit time.

Study protocol

We evaluated lipid profile and endothelial function before (T0), immediately after (T1), 24h after (T2) and 72h after (T3) a lipoprotein apheresis. All subjects were evaluated at morning in a quiet room, starting with blood sampling. Subsequently, medical questionnaire and physical examination were performed. After resting comfortably for at least 15 minutes in supine position, a single operator performed blood sampling and peripheral arterial tonometry; subsequently, patients underwent a lipoprotein apheresis. After the procedure, blood samples were taken from patients and endothelial function assessment performed by PAT. Successively, evaluation of lipid profile and PAT assessment were performed three days after lipoprotein apheresis.

Informed consent

Informed consent was obtained from all patients, according to the Declaration of Helsinki.

Clinical assessment

Clinical evaluation included: medical questionnaire; physical examination, in order to collect variables such as heart rate, blood pressure values, height, weight, body mass index (BMI), waist circumference; 12-lead electrocardiogram at rest. The presence of cardiovascular risk factors (CRFs) was assessed in each subject, according to current guidelines: hypertension (systolic blood pressure >140 mm Hg,

diastolic blood pressure >90 mmHg, according to the guidelines of European Society of Hypertension/European Society of Cardiology, or taking an antihypertensive treatment); dyslipidaemia (according to the Third report of the National Cholesterol Education Program (NCEP-III) and EAS 2012, or taking lipid-lowering medication]; diabetes mellitus (treated with an oral hypoglycaemic agent, insulin, or both, or having fasting glucose levels >126 mg/dl, in agreement with the American Diabetes Association); family history of CAD (having first- or second- degree relatives with premature cardiovascular disease); and smoking habit. We considered traditional cardiovascular risk factors: dyslipidaemia, or LDL≥70mg/dl; hypertension, or systolic blood pressure ≥140 and diastolic blood pressure ≥90, for non-diabetics and 120 SBP and 80 DBP for diabetics; BMI≥25; diabetes; smoke habits.

Lipoprotein apheresis procedure

Patients on regularly weekly or biweekly lipoprotein apheresis were treated with the FDA-approved Heparin-induced Extracorporeal LDL Precipitation (H.E.L.P.) (Plasmat Futura, B.Braun, Melsungen, Germany) technique [10]. The anticoagulation was performed by an initial heparin bolus (20 IU heparin/Kg body weight), followed by heparin continuous infusion. Antecubital veins served as blood access. The mean plasma volume treated per session was approximately 3 or 4 L, according to American Society for Apheresis (ASFA) guidelines [10].

Reactive hyperaemia by peripheral arterial tonometry

Endothelial function was measured by peripheral arterial tonometry (PAT); PAT values were expressed as natural logarithm of reactive hyperaemia index values (ln-RHI), according to the previously described procedure [11]. PAT signals were obtained using the EndoPAT 2000 device (Itamar Medical LTD Caesarea, Israel), a non-invasive technique offering a beat-to-beat plethysmographic recording of the finger arterial pulse-wave amplitudes by digital pneumatic probes, which has been largely validated and used to evaluate cardiovascular risk [12-15]. An extensive description of this method and of the analyses algorithm was provided elsewhere [16]. According to literature [17], Ln-RHI>0.4 was considered as cut off for normal values. We performed PAT assessment at T0, T1, T2 and T3.

Blood sampling

Venous blood samples at T0 were collected before single apheretic procedure, in the morning, after an overnight fasting ; blood samples at T1 were collected immediately after the apheretic procedureT1. Venous blood samples at T2 and T3 were collected 24h (T2) and 72h (T3) after procedures, respectively, in the morning, after an overnight fasting. Venous blood samples were collected from the antecubital vein into evacuated plastic tubes (Vacutainer).

Flow cytometric analysis

Endothelial Progenitor Cells (EPC) and Circulating Endothelial Cells (CEC) evaluation was assessed by flow cytometry as previously described method [18]. Circulating EPCs were identified through their expression of CD34, KDR, and CD133 and were considered as EPC cells CD34+/KDR+, CD133+/KDR+ and CD34/CD133+/KDR+. CECs were defined as cells forming a cluster with low side scatter and low-to-intermediate CD45 staining and positive for CD34+, CD133+, and CD34+/CD133+. The intra-assay coefficient of variation of the EPC measurement was 7.8%. The intra-observer and inter-observer variations of our method showed an intra-class correlation coefficient of 0.97 and 0.92, respectively.

We performed EPC and CEC assessment at T0, T1 and T2.

Biochemical parameters

Fibrinogen was measured by clotting assay and high sensitive C reactive protein

(hs-CRP) by a high-sensitivity nephelometric assay (Dade Behring GmbH, Marburg, Germany). Lp(a) was measured by an immune-nephelometric method (LPAX IMMAGE Beckman Coulter); values ≥500 mg/L were considered abnormal. Lipid profile analyses and complete blood count were performed by standard methods.

Statistical analysis

Database construction and statistical analysis were performed with SPSS (Statistic Package for Social Sciences, Chicago, USA) for Windows (Version 19). Categorical variables were expressed as frequencies and percentages; analysis of data distribution was evaluated by chi-square test (statistical significance was for $p<0.05$). Continuous variables were expressed as mean±SD.

Continuous variables were compared using Analysis of Variance test. The non-parametric Mann-Whitney and Kruskall-Wallis tests were used for analysis of unpaired data. Correlation analysis was measured by using the Spearman's correlation test. A p-value <0.05 was considered statistically significant.

Results

Characteristics of study population

Characteristics of study population were listed in table 1.

No current smokers were found in the study group. All subjects were taking one or more anti-hypertensive drugs with a good pressure control. Only one subjects was diabetic, with an optimal glycaemic control (Hb1Ac = 5.2%, fasting glucose = 0,95 g/L). One subject was intolerant to statins.

Patients did not show any adverse effect related to lipoprotein apheresis; procedures were performed according to guidelines recommendations (see table 2 for biochemical parameters pre- and post- procedure). Markers of liver and renal function appeared within normal limits at the different samples.

Table 1: Characteristics of study population.

Clinical characteristics	
Male, n, (%)	5, (62.5%)
Age, mean (range), years	63.29, (12)
SBP, mean±SD mmHg	112.12 ± 18.02
DBP, mean±SD mmHg	76.57 ± 7.50
BMI, mean±SD (Kg/m^2)	23.25 ± 1.67
BMI ≥25(Kg/m^2), n (%)	0 (0%)
Diabetes, n, (%)	1, (12.5%)
Hypertension, n, (%)	8, (100%)
Peripheral Arterial Disease, n, (%)	8 (100%)
Family history for CAD	8 (100%)
Pharmacological therapies	
Anti-hypertensive therapy, n, (%)	8, (100%)
Ace-inhibitors	5, (62.5%)
ATII blockers	3, (37.5%)
Beta-blockers	3, (37.5%)
Ca++-antagonists	2, (25%)
Diuretics	4, (50%)
Statins, n, (%)	7, (87.5%)
PUFA, n, (%)	3, (37.5%)
Antiplatelets, n, (%)	8, (100%)
OAT, n, (%)	0, (0%)
Oral hypoglycemic agents, n, (%)	2, (25%)
Nitrates, n (%)	2, (25%)

SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; BMI: Body Mass Index; CAD: Coronary Artery Disease; ATII blockers: Angiotensin II Blockers; PUFA: Polyunsaturated Fatty Acids; OAT: Oral Anticoagulant Therapy.

Biochemical parameters during lipoprotein apheresis

Biochemical parameters at different times were described in table 2.

At baseline, all patients showed LDL-c>70 mg/dl, in disagreement with current guidelines. As expected, LDL-c, triglycerides and Lp(a) values were significantly lower after procedure. At T1, we found a significant reduction in LDL-c (74%), triglycerides (35%) and Lp(a) (88%) values; this reduction persisted at T2, showing a value of 29%, 84%, 41% of baseline, respectively (Table 2). Reduction in lipid markers was documented in all patients. Regarding inflammatory markers, fibrinogen and hs-CRP values were decreased at T1, T2, and T3 in comparison to the baseline.

Peripheral arterial tonometry and Flow Cytometric analyses

Peripheral arterial tonometry values before lipoprotein apheresis were within normal limits in all patients.

Ln-RHI values of study population were lower than values found in primary prevention subjects, as reported in previously published data [18,19].

We found a reduction in Ln-RHI after procedure (Table 2), the difference between T2 and T0 was statistically significant. The reduction in Ln-RHI values after apheresis was documented in all patients; in particular, two subjects showed an Ln-RHI<0.4 at T1, and four subjects showed abnormal values at T2.

At T3, PAT values were increased significantly in comparison to T1 and T2, with a median value higher than at T0.

Regarding EPC count, we showed a progressive increase at T2 in comparison to baseline. In detail, Cd34+/Kdr+ and Cd133+/Kdr+ showed a minimum increase in median values at T1, and a higher increase at T2, in comparison to baseline. Differences in Cd34+/133+/Kdr+ values at different times were not statistically significant. A significant reduction in CEC count at T2 in comparison to T0 was found.

A significant correlation between Cd34+/Kdr+ and Cd133+/Kdr+ count (r=0.98, p<0.0001), and Cd34+/Cd133+/Kdr+ count (r=0.82, p<0.0001) was found; similarly, Cd133+/Kdr+ and Cd34+/Cd133+/Kdr+ count correlated significantly (r=0.97, p<0.0001).

Table 2: Biochemical parameters, endothelial function values and different clusters of endothelial progenitor cells at each time.

Biochemical parameters	*T0*	*T1*	*T2*	*T3*	*P*(T0-T1)*	*P*(T1-T2)*	*P*(T0-T2)*	*P*(T0-T3)*
Hb, mean±SD, g/dl	12.9±1.7	-	-	-	-	-	-	-
Hct, mean±SD, %	39.2±3.9	-	-	-	-	-	-	-
Fibrinogen, mean±SD, mg/dl	275.6±173.2	90.0±18.7	170.3±36.7	220±69.7	**0.016**	0.3	**0.02**	0.2
Creatinine, mean±SD, mg/dL	0.83±0.24	-	-	-	-	-	-	-
AST, mean±SD, U/L	24.0±9.8	-	-	-	-	-	-	-
ALT, mean±SD, U/L	24.0±9.2	-	-	-	-	-	-	-
GGT, mean±SD, U/L	12.5±7.8	-	-	-	-	-	-	-
CPK, mean±SD, U/L	167.3±58.0	-	-	-	-	-	-	-
LDL-c, mean±SD mg/dl	100.75±38.85	26.5 ± 9.89	28.6±10.2	42.4± 18.2	**0.041**	0.8	**0.042**	**0.049**
HDL-c, mean±SD mg/dl	49.5±2.89	39.0±9.4	42.3±7.4	44.1± 10.8	0.09	0.7	0.09	0.1
Triglycerides, mean±SD mg/dl	78.5±38.6	50.75±5.2	65.9±23.5	76± 31.3	0.2	0.2	0.3	0.3
C-reactive protein, mean±SD	0.02 ± 0.02	0.01 ± 0.02	0.01±0.01	0.01± 0.02	0.5	0.8	0.08	0.09
Lipoprotein(a) mean±SD, mg/L	1450.25±220.1	310.75±160.26	590.8±182.34	612.2±150.2	**0.005**	0.4	**0.01**	**0.04**
Peripheral arterial tonometry								
Ln-RHI, mean±SD	0.72 ± 0.29	0.57 ± 0.21	0.43±0.24	0.91± 0.18	**0.05**	0.1	**0.006**	0.1
Flow cytometric analyses								
CD133+/KDR+	7.14±10.08	3.71±4.82	9.00±5.77	-	0.3	**0.018**	0.7	-
CD34+/KDR+	7.14±11.82	3.71±4.82	9.00±5.77	-	0.3	**0.018**	0.7	-
CD133+/34+/KDR+	6.71±10.74	3.29±5.02	3.71±4.82	-	0.3	0.4	0.6	-
CEC	23.86±12.39	30.43±30.39	12.00±8.85	-	0.6	0.2	**0.024**	-

*P values between T0-T1, T1-T2 and T0-T3; bold values represent statistically significant values.

Discussion

We examined a secondary prevention population with high Lp(a) levels, undergoing chronic lipoprotein apheresis treatment for at least 1 year, according to ASFA guidelines. All subjects underwent the same procedure, not experimenting adverse effect during the study period. In this study, we observed that each single procedure was associated to a significant improvement in lipid profile, and, at 24h and 72h after procedures, we found an improvement in endothelial function expressed by an increase in PAT values and EPC levels, and by a reduction in CEC. Scarce evidences documented the acute effect of apheresis on microvascular function, as assessed by PAT; in particular, Lu et al. [20], reported a lack in improvement in endothelial function after acute apheretic procedure, confirming our data. Moreover, Patschan et al. [8] reported that did not show an increase in total EPCs immediately after apheresis treatment in hyperlipidemic patients.

Recent evidences showed that the global improvement in endothelial function resulting from the procedure was documented within 20 hours after the treatment. In particular, Ramunni et al. [9], showed an increase in EPCs levels at 24 hours after lipoprotein apheresis, suggesting that this variation was relative to the mobilization of the dormant pool. Mellwig et al. [21-23], documented the improvement of endothelial function within 20 hours after procedure in patients with homozygous and therapy-resistant hypercholesterolemia treated with the HELP technique.

Several mechanisms responsible for the beneficial pleiotropic effects of lipoprotein apheresis on endothelial function could involve the optimization of lipid profile, the reduction of oxidative stress, the benefit on vascular share stress and rheology, and the improvement on inflammatory homeostasis at micro- and macrovascular districts [4,21], in particular, these different pathways could finally affect the count of various progenitor cells populations. In addition, drug therapies ongoing at the time of the study are known to positively modulate endothelial function. The sum of these variables could explain the seemingly good endothelial function at baseline, despite the very high cardiovascular risk.

The positive correlation found between the percentage of increase of Ln-RHI and Lp(a) values may express the more beneficial results in patients with higher basal Lp(a) values; the procedure may result in a global improvement of vascular function reducing Lp(a) in patients who are chronically more exposed to its damage. This cumulative positive effect on endothelial function is probably reinforced at each lipoprotein apheresis, conferring a protective effect on vascular function. Furthermore, we found a significant reduction in fibrinogen and hs-CRP levels, attesting a positive affection on inflammatory system. At our knowledge, this is the first work evaluating instrumental and cellular markers of endothelial function, and showing the 72h effects of lipoprotein apheresis.

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