Changes in Myocardial Vasoreactivity After Drastic Reduction of Plasma Fibrinogen and Cholesterol: a Clinical Study in Long-term Heart Transplant Survivors Using Positron Emission Tomography

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Background: Given the central importance of the microvasculature in heart transplant recipients, we investigated the possibility of increasing cardiac perfusion after reduction of low-density lipoprotein (LDL)-cholesterol, lipoprotein (a), C-reactive protein (CRP) and fibrinogen plasma levels after apheresis treatment in transplanted patients.

Methods: Ten long-term heart transplant recipients were examined with positron emission tomography (PET) to measure myocardial perfusion before and after a single heparin-mediated extracorporeal LDL/fibrinogen precipitation (HELP)-apheresis treatment. PET studies were performed the mornings before and after the apheresis treatment. Myocardial blood flow at rest and during adenosine-induced hyperemia was measured using ^{13}N-ammonia.

Results: HELP-apheresis reduced the plasma levels of LDL-cholesterol, lipoprotein (a) and C-reactive protein by 48% ($p < 0.001$), fibrinogen by 42% ($p = 0.02$), plasma viscosity by 14% ($p = 0.004$) and erythrocyte aggregation by 28% ($p < 0.02$). Osmolality (<1%) and hematocrit (<2%) remained stable. A single apheresis treatment increased median corrected rest flow by 17.5% ($p = 0.007$) and median hyperemic flow by 27% ($p = 0.02$). Median coronary flow reserve increased by 8.1% ($p = 0.09$). Hyperemic flow after adenosine infusion increased plasma vascular endothelial growth factor levels only before HELP-apheresis (+60%), indicating better ischemic tolerance after apheresis ($p = 0.01$).

Conclusions: Myocardial perfusion in transplanted hearts increases significantly after single HELP-apheresis treatment. The present study is only a proof of concept, providing complementary evidence to clinical long-term studies showing that cholesterol reduction either with statins and/or apheresis improves heart transplant outcome. J Heart Lung Transplant 2005;24:2022–30. Copyright © 2005 by the International Society for Heart and Lung Transplantation.

It is well recognized that the flowing blood interacts with the arterial vessel wall in an interdependent manner. Changes in blood composition affect vasotonus control, a circumstance that has been utilized for the treatment of arteriosclerosis. The most relevant example is low-density lipoprotein (LDL)-cholesterol, the reduction of which has been shown to decrease coronary vascular resistance.[1–3] This effect seems to be mediated through the improvement of endothelial function via activation of nitric oxide.[4] Interventional studies using statins later confirmed that the main benefit of cholesterol reduction did not originate from appreciable plaque regression, but from the reduction of coronary vascular resistance and other plaque-stabilizing effects.

Interestingly, LDL-cholesterol reduction improves coronary vasomotion irrespective of the method used, such as diet, fish-oil, statins or LDL apheresis.[1–3,5–7] All of the different treatment modalities are effective, and they differ only in intensity and time period until vasomotion improves. Statins, for instance, produce relevant vasomodulatory effects only between 6 weeks and 6 months after beginning of treatment.[1,8,9] By contrast, the vasomodulatory effects of LDL apheresis can be measured already during treatment and shortly

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afterward,[7,10-12] and these effects might be related to the fact that the full cholesterol reduction is achieved simultaneously. Notably, these effects are not restricted to the heart, but equally extend to the brain[11] and the peripheral vasculature.[10]

In an attempt to improve the poor outcome of heart transplant patients with impending or overt cardiac allograft vasculopathy (CAV), single transplant centers (mainly Munich and Duisburg) combined statins with regular application of heparin-mediated extracorporeal LDL/fibrinogen precipitation (HELP)-apheresis in select high-risk patients. To maximize the "anti-atherogenic" potential, the use of apheresis[13-16] was added, because HELP-apheresis has complementary "anti-thrombotic" and "anti-inflammatory" properties, allowing for a long-lasting reduction of plasma fibrinogen and its precursors as well as C-reactive protein (CRP) by >50%. However, although blood levels of cholesterol, fibrinogen, CRP, lipoprotein (a) and other pro-thrombogenic blood compounds like plasminogen activator inhibitor-1 (PAI-1) and tissue factor[17-21] are elevated in transplant patients, and the incidence of CAV increases with their plasma concentrations, only cholesterol reduction using statins has been shown to be therapeutically effective for CAV.[22,23]

The vasomodulating and plaque-stabilizing effects of HELP-apheresis as a hemorheology-improving tool in CAV have not been studied, although first clinical studies indicate that this treatment is extremely promising,[14-16] with a 10-year survival rate of 82% for CAV patients.[16] Therefore, an understanding of the dynamic interactions between blood consistency and coronary blood flow in the transplanted heart in relation to this treatment is important. To learn more about the relevance of the coronary risk factors such as fibrinogen, plasma viscosity, red cell aggregation and cholesterol for coronary vasomodulation in heart transplant patients, we performed the present study.

METHODS

Objective and Study Design

The primary objective of this study was the comparison of myocardial blood flow before and shortly after apheresis treatment. Secondary objectives were the analysis of the hemorheologic consequences and the possible role of mediators such as nitric oxide or vascular endothelial growth factor (VEGF) in this setting. The study protocol is outlined in Figure 1. First, a baseline PET examination was performed under rest conditions and after adenosine infusion. At that time, patients had elevated plasma levels of LDL-cholesterol, lipoprotein (a), fibrinogen and CRP.

Second, a PET examination was repeated following the same protocol after a single HELP-apheresis treatment. At that time, the patients had approximately 50% lower plasma cholesterol, fibrinogen, lipoprotein (a) and CRP levels.

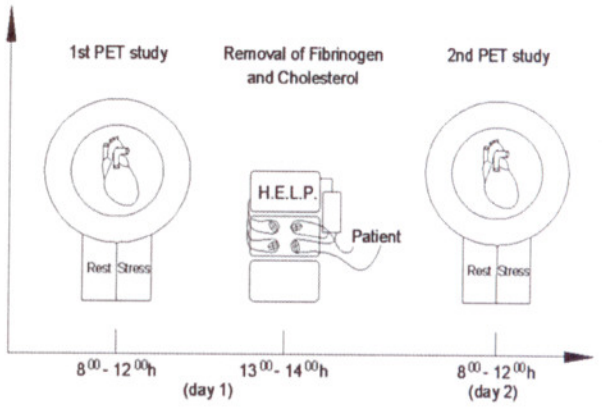

Figure 1. The first PET study was done in the morning of Day 1. In the afternoon the patient was treated with extracorporeal apheresis, and on the next morning the second PET study was done following the same protocol as the first.

Third, the data acquired from both PET examinations were blinded and analyzed separately by 2 investigators.

Patients

The study participants (9 men, 1 woman) were long-term survivors after orthotopic heart transplantation (HTx), and were regularly treated with HELP-apheresis for their pronounced atherosclerotic risk constellation, as outlined in Table 1. Continuous apheresis was repeated in weekly to bi-weekly intervals (average 11.5 days), and this treatment started 2.7 years after HTx. At the time of the study (mean interval of 5.6 ± 2.3 years after HTx), 6 patients showed angiographic signs of CAV as defined by Gao et al.[24] One patient had a prominent calcified plaque in his left arterior descendent artery and the remaining 3 patients had normal angiographic findings.

Post-transplant medications are listed in Table 1. Concurrent medication consisted of aspirin (n = 9), furosemide (n = 9), diltiazem (n = 6) and/or enalapril (n = 6). Statins (simvastatin 20 mg/day [n = 9] and 15 mg/day [n = 1]) were discontinued 1 week before and during the study period. Patients also refrained from drinking caffeine-containing beverages or eating nitrate-containing food. All patients were non-smokers. The ethics committee of the faculty approved the study protocol and the patients provided written informed consent before the study was initiated.

PET Studies

Dynamic PET measurements were performed with a CTI/ECAT 951R/31 scanner (Siemens/CTI, Knoxville, TN) using a standard protocol.[25,26] After positioning,

Table 1. Characteristics of the Study Patients

Variable	
Gender (M/F)	9/1
Recipient age (years)	55 ± 6
Donor age (years)	30 ± 13
Recipient body mass index (kg/m^2)	24.4 ± 1.5
Arterial hypertension after HTx (Y/N)	9/1
Diabetes mellitus after HTx (Y/N)	2/8
Former smoking (Y/N)	9/1
Positive family history for CAD (Y/N)	7/3
Hypercholesterolemia[a] after HTx	10/0
Lipoprotein(a) increase (>30 mg/dl) after HTx (Y/N)	8/2
Fibrinogen increase (>4 g/l) after HTx (Y/N)	7/3
Reason for HTx (ICM/DCM)	10/0
CAD after HTx (Y/N)	7/3
Cytomegalovirus infection after HTx (Y/N)	1/9
Time since HTx (years)	5.6 ± 2.3
Start of HELP–apheresis post-HTx	2.7 ± 1.7
Frequency HELP–apheresis treatment (days)	11.5 ± 3.4
Immunosuppressive medication	
Cyclosporine (Y/N)	8/2
Azathioprine (Y/N)	2/8
Prednisolone (Y/N)	10/0
Mycophenolate (Y/N)	1/9

CAD, coronary artery disease; DCM, dilated cardiomyopathy; HELP, heparin-mediated extracorporeal low-density lipoprotein/Fibrinogen precipitation; HTx, heart transplant; ICM, ischemic cardiomyopathy; N, no; Y, yes.

[a]Definition of hypercholesterolemia refers to the baseline LDL-cholesterol before the initiation of regular HELP–apheresis; cholesterol levels before treatment were, on average, 191 ± 44 mg/dl.

a transmission scan was obtained over 15 minutes for attenuation correction of emission data. Consequently, for the rest study a bolus of 20 mCi of ^{13}N-ammonia was infused intravenously over 30 seconds. The dynamic PET data acquisition consisted of 21 frames of varying duration (12 × 10 seconds, 6 × 30 seconds, 3 × 300 seconds). After decay of the initial activity (i.e., after 50 minutes) the stress study began, with intravenous infusion of adenosine over 5 minutes (0.14 mg/kg body weight per minute). Another 20-mCi dose of ^{13}N-ammonia was administered 2 minutes into the adenosine infusion, and dynamic imaging was started at the same time. Heart rate and blood pressure were continuously registered throughout imaging, and the rate-pressure product (RPP; heart rate [min^{-1}] multiplied by systolic blood pressure [mm Hg] and a correction factor) was calculated as a measure of cardiac work. Attenuation-corrected transaxial images were reconstructed using a Hanning filter (cutoff: 0.3 cycle/bin). Subsequently, images were re-oriented along the long cardiac axis, and regions of interest for blood pool and myocardial segments were defined using previously validated software.[26,27] Motion-corrected time-activity curves were generated, and quantification of regional myocardial blood flow by fitting of time-activity curves was done according to a previously validated 3-compartment tracer kinetic model.[28]

Heparin-mediated Extracorporeal LDL/Fibrinogen Precipitation (HELP)-Apheresis

The HELP-apheresis system is an extracorporeal plasma treatment designed for maximal treatment of atherosclerosis and related clinical complications.[29] Among the various apheresis approaches, HELP-apheresis allows for simultaneous removal of several major proatherogenic blood compounds. It reduces 50 ± 10% of LDL-cholesterol, lipoprotein (a), CRP, fibrinogen, prothrombin, von Willebrand factor and Factor XIII, and 30% to 60% of Factor VIII, IX, X, XI, XII, serum vascular cell adhesion molecule-1 (sVCAM-1), serum inter-cellular adhesion molecule-1 (sICAM-1) and soluble tissue factor levels. In addition, the HELP-apheresis system does not remove protective blood compounds such as high-density lipoprotein (HDL)-cholesterol or immunoglobulins.[29] The system uses a Plasmat-Secura apparatus (B. Braun AG, Melsungen, Germany), and can deliver an elimination rate of 100% for the aforementioned plasma compounds. The regulator for reduction of the plasma compounds is the quantity of plasma being filtered, which was a standard quantity of 3 liters in this study. The system has been described in detail elsewhere.[29] Regarding the safety precautions, no relevant complications or adverse effects of this treatment have been reported to date in at least 200,000 treatments in 1,000 patients.

Laboratory Methods

The blood samples were drawn before each PET study and during adenosine infusion. Cholesterol and triglycerides were measured by enzymatic colorimetric assays (CHOD-PAP method and GPO-PAP method) from Roche Diagnostics (Mannheim, Germany) using a Hitachi 705 device. LDL and HDL were determined by standard precipitation techniques (Immuno Heidelberg, Germany), and lipoprotein (a) by a turbidimetric assay (Greiner, Flacht, Germany). Fibrinogen was measured according to Clauss[30] in an STA apparatus with reagents from Roche. Ultrasensitive CRP was measured by immunturbidimetry with reagents also from Roche.

Plasma catecholamines were measured electrochemically (Model 460 Electrochemical Detector, Waters) after separation with high-performance liquid chromatography (HPLC; AS 2000 A-Autosampler, Merck, Darmstadt, Germany). Osmolality was determined using a micro-osmometer (3MO Plus, Fa. Dinkelberg, Neu-Ulm, Germany), based on the principle of freezing-point depression. Plasma viscosity (2.2 ml of potassium-ethylene-diamine tetraacetic acid [K-EDTA] plasma) was analyzed with a capillary tube plasma viscosimeter (Fresenius, Oberursel, Germany) according to the

Table 2. Changes in Lipoproteins, Fibrinogen and Hemorheologic Variables During the Study

Variable		1st PET Study Baseline	2nd PET Study After Apheresis	Difference 1st vs 2nd PET	Desired Levels‡
LDL-Cholesterol	[mg/dl]	191 ± 44	99 ± 22*	[−48%]	<100
Lipoprotein (a)	[mg/dl]	63 ± 29	33 ± 17*	[−48%]	<30
C-reactive protein	[mg/dl]	0.95 ± 1.31	0.499 ± 0.70*	[−48%]	<0.5
Fibrinogen	[g/l]	4.65 ± 0.95	2.70 ± 0.76*	[−42%]	<300
sVCAM-1	[ng/ml]	832 ± 312	570 ± 242*	[−31%]	<714
sICAM-1	[ng/ml]	368 ± 123	309 ± 120*	[−16%]	<306
Plasma Viscosity	[mPa]	1.36 ± 0.11	1.17 ± 0.05*	[−14%]	<1.24
Erythrocyte Aggregation	[au]†	27.1 ± 5.4	19.5 ± 7.9*	[−28%]	<24.0
Hematocrit	[Vol%]	36.2 ± 5.1	35.6 ± 5.4	[−1.7%]	<0.52
Osmolality	[mmol/kg]	304 ± 7	301 ± 5	[−1.0%]	<295

PET, positron emission tomography; sVCAM-1, serum vascular cell adhesion molecule-1; sICAM, serum intracellular adhesion molecule-1.

*P-value < 0.05 for the comparison of the variable after apheresis vs baseline.

†[au] is the abbreviation for arbitrary units.

‡Desired levels refer to the upper limit of the normal ranges, or recommendations deduced from intervention studies for LDL-cholesterol and fibrinogen.

method of Jung et al.[31] Erythrocyte aggregation was measured according to Schmidt-Schönbein et al.[32] Plasma nitric oxide was determined photometrically with a commercial kit (R&D Systems, Minneapolis, MN); the vascular endothelial growth factor (VEGF) concentration was obtained with a commercial kit using an enzyme-linked immunoassay (ELISA) technique (R&D Systems).

Statistical Analysis

Two independent investigators analyzed the PET studies. The PET scans were assessed in a blinded manner. The interobserver correlation for rest and stress myocardial blood flow (MBF) was good ($r = 0.892$, Fisher's R-Z test). The mean difference between both observers was 0.003, with a corresponding p-value of 0.9563 (paired Student's t-test). In the next section, the means are shown for both investigators. Continuous variables were usually expressed as mean ± standard deviation, and median concentrations and ranges were given for variables not normally distributed. For comparison of the continuous variables before and after HELP-apheresis, Wilcoxon's matched-pair log-rank statistic was used. $p < 0.05$ (2-tailed test) indicated statistical significance.

RESULTS

Comparison of Laboratory Variables

Plasma concentrations of LDL-cholesterol (LDL-C), lipoprotein (a), fibrinogen and CRP before and after apheresis are outlined in Table 2. The baseline concentration of LDL-cholesterol was elevated considerably (191 ± 44 mg/dl), as were the concentrations of lipoprotein (a) (63 ± 29 mg/dl), CRP (0.95 ± 1.31 mg/dl) and fibrinogen (4.65 ± 0.95 g/liter). HELP-apheresis significantly reduced the concentrations to almost normal levels. On the following day before the second PET study the concentrations were 48% lower for LDL-C ($p < 0.002$), lipoprotein (a) ($p < 0.002$) and CRP ($p < 0.002$) and 42% lower for fibrinogen ($p < 0.02$) (Table 2). The different elimination rate for lipoproteins and fibrinogen reflects the new slower rate of lipoprotein synthesis.

The hemorheologic variables are also summarized in Table 2. The baseline levels for plasma viscosity (PV) and erythrocyte aggregation were both elevated: plasma viscosity was 1.36 ± 0.11 mPa s (approximate normal: 1.24 ± 0.1 mPa s) and erythrocyte aggregation was 27.1 ± 5.4 arbitrary units (approximate normal range 20.0 ± 3.7 arbitrary units). The day after HELP-apheresis, when the second PET study took place, plasma viscosity was 14% ($p < 0.004$) lower and the erythrocyte aggregation 28% ($p < 0.02$) lower. Notably, neither hematocrit nor osmolality were reduced after apheresis: the difference for both variables was <2%; thus, the rheologic effect is not explained by hemodilution (Table 2), but mainly by removal of fibrinogen.

Plasma noradrenaline, adrenaline and dopamine were measured before and after each PET study. Because cardiac denervation after transplantation hinders the neuronal liberation of catecholamines, a partial compensation by plasma catecholamines is to be expected. We found the noradrenaline concentration elevated during both PET studies: 2.8 ± 1.0 vs 3.6 ± 1.4 nmol/liter (normal range: 1.1 to 1.7 nmol/liter). Conversely, the adrenaline and dopamine levels were slightly below the normal ranges, but without significant differences between study days.

Comparison of Myocardial Blood Flow During Both PET Studies

A comparison of MBF before and after HELP-apheresis is shown in Table 3. Before apheresis, the median absolute resting MBF was 0.965 ml/g/min. One day after

Table 3. Hemodynamic Response to Adenosine Infusion

Variable		1st PET Study Before Apheresis	2nd PET Study After Apheresis	Difference of Median (%)	Wilcoxon test *p*-Value
Baseline rest flow [ml/g/min]	median:	0.965	1.127	+16.8%	($p = 0.14$)
	mean:	0.979 ± 0.313	1.107 ± 0.324		
	range:	0.506–1.376	0.550–1.700		
Corrected rest flow [mL/g/min]	median:	0.692	0.813	+17.5%	($p = 0.007$)
	mean:	0.681 ± 0.140	0.869 ± 0.242		
	range:	0.445–0.872	0.555–1.447		
Stress flow [ml/g/min]	median:	2.019	2.573	+27%	($p = 0.047$)
	mean:	1.947 ± 0.612	2.424 ± 0.590		
	range:	0.934–2.923	1.130–3.144		
CFR	median:	2.113	2.284	+8.1%	($p = 0.09$)
	mean:	2.088 ± 0.684	2.281 ± 0.670		
	range:	1.072–3.573	1.441–3.598		
Corrected CFR	median:	2.813	3.078	+9.4%	($p = 0.57$)
	mean:	2.950 ± 1.099	2.901 ± 0.795		
	range:	1.431–5.342	1.624–3.884		
Mean arterial pressure* [mmHg]	mean:	85 ± 7	100 ± 12	+17.6	($p = 0.04$)
Minimal coronary resistance† [mmHg/ml/g/min]	mean:	0.50 ± 0. 1	0.45 ± 0.15	−10.0%	($p = 0.09$)
Rate pressure product [bpm × mmHg]	mean:	11.6 ± 3.0	11.1 ± 2.8	−4.3%	($p = 0.07$)

CFR, coronary flow reserve.

*Mean arterial pressure was calculated as diastolic blood pressure + 1/3 (systolic blood pressure − diastolic blood pressure).

†Minimal coronary resistance was defined as mean arterial pressure divided by stress flow.

apheresis, the median absolute resting MBF was 1.127 ml/g/min. The difference between days amounts to +16.8% (Wilcoxon test, comparison of medians: $p = 0.14$), with 5 patients having higher flow rates than the day before.

When one compares the change in resting flow irrespective of differences in rate-pressure product between study days (11.6 ± 3.0 vs 11.1 ± 2.8 beats/min × mm Hg), usually the "corrected resting flow" is given, defined as absolute resting flow divided by the rate-pressure product (heart rate × systolic pressure) and multiplied by a standardization factor of 8,000: Before apheresis, the median corrected resting MBF was 0.692 ml/g/min and, after apheresis, the median corrected resting MBF averaged 0.813 ml/g/min. The difference was +17.5% ($p = 0.007$), with 8 patients having higher flow rates after HELP-apheresis, showing more clearly the effect of treatment.

Before HELP-apheresis, the adenosine-induced median hyperemic flow peaked at 2.019 mg/dl (Table 3). One day after HELP-apheresis, the hyperemic flow of the heart transplant patients increased to a median of 2.573 mg/dl. The difference between both days upon comparing median levels showed a significant increase of 27% ($p = 0.047$), and 8 patients exhibited higher flow rates on the second day. Likewise, the lowest hyperemia flow was observed in a patient with a CAV narrowing of all major coronary arteries by up to 90% to 95%, and the highest flow was seen in a patient without CAV.

The global coronary flow reserve (CFR), calculated as mean hyperemic flow divided by mean rest flow, increased from 2.088 ± 0.684 before apheresis to 2.281 ± 0.670 on the day after apheresis. Again, upon comparison of median levels, this represents an increase from 2.113 to 2.284 (i.e., 8.1%), which indicates a trend ($p = 0.09$), although not statistically significant.

Assuming a CFR of 3.5 as being the lower end of normal, only 1 study patient reached this level. If a CFR of 3.0 is considered the lower end, then at least 3 patients were above this level before and 6 patients after apheresis.

Comparison of Hemodynamic Determinants During Both PET Studies

Heart rate at baseline was slightly elevated on both PET study days (82 vs 81 beats/min), which is a common finding after cardiac denervation. Blood pressure was 140/79 mm Hg before and 136/76 mm Hg after HELP-apheresis. This difference was not significant but could explain the difference in the rate-pressure product, which is a formula that corrects for basal heart work by multiplying systolic blood pressure by pulse at baseline: 11.6 ± 3.0 vs 11.1 ± 2.8 mm Hg/min ($p = 0.07$).

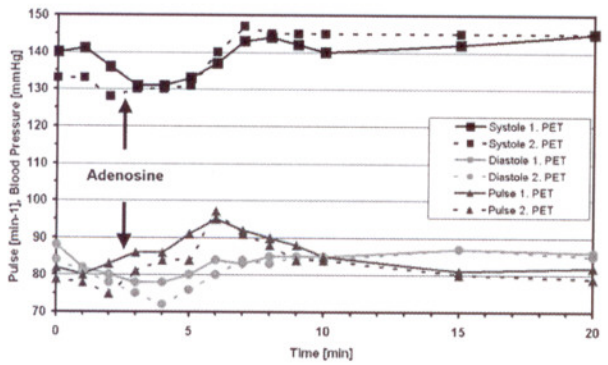

Figure 2. Changes in blood pressure and heart rate after infusion of adenosine to mimic a stress situation with maximal dilation of the coronary arteries.

The changes in the hemodynamic parameters during the hyperemic studies with adenosine are shown in Figure 2. To induce maximal vasodilation, adenosine was infused from Minutes 3 to 8. Notably, the pulse rate of heart transplant patients was less pronounced upon infusion. The pulse rate increased from 80 beats/min at minimum to a peak of 98 beats/min in the sixth minute. This could be observed equally during both PET studies. Similarities could also be observed in the blood pressure curves on both days (Figure 2). Adenosine infusion provoked first a paradoxical decrease of both systole and diastole, a phenomenon apparently related to denervation, as described earlier. Also, the final increase in systole (from a minimum of 128 mm Hg to a maximum of 148 mm Hg) and in diastole (from a minimum of 72 mm Hg to a maximum of 85 mm Hg), with the peak corresponding to the end of adenosine infusion, was less pronounced than expected in comparison to similar studies in non-transplanted patients.

The mean myocardial blood flow in the 3 main epicardial arteries did not differ to a statistically significant degree . Regional perfusion was rather homogeneous: left anterior descendent (LAD), 0.995 ± 0.314; right coronary artery (RCA), 1.085 ± 0.335; and left circumflex artery (LCX), 1.027 ± 0.305 ml/g tissue/min. Corresponding to global MBF, resting perfusion increased homogeneously the day after apheresis. Similar results were seen for the hyperemic studies after adenosine infusion. CFRs varied between 1.8 and 2.0 before apheresis and between 2.2 and 2.3 after apheresis.

Comparison of Vasoactive Mediators During PET Studies

We measured plasma nitric oxide, vascular endothelial growth factor (VEGF) and brain natriuretic peptide (BNP) to determine whether the increment in myocardial blood flow was linked to changes in the vasoactive mediators. Plasma nitrate/nitrite levels did not change upon adenosine infusion before (median at rest: 99 μmol/liter; median with stress: 96 μmol/liter; p = not significant [NS]) or after (median at rest: 89 μmol/liter; median with stress: 78 μmol/liter; p = NS) HELP-apheresis. Also, the concentrations were significantly higher than those of internal controls, which consisted of 13 HELP-apheresis patients with native CAD (61 ± 28 μmol/liter). BNP decreased by 16% after HELP-apheresis.

By contrast, plasma VEGF levels reacted differently before and after HELP-apheresis upon adenosine infusion (Figure 3). Before HELP-apheresis, plasma VEGF increased from a median concentration of 76 to 121 ng/ml, which is a significant increase of 60% (p = 0.05). After apheresis, baseline VEGF concentration was lower (58 ng/ml) and adenosine infusion was not followed by a further increase in concentration (31 ng/ml) (Figure 3). During both PET examinations there was considerable intra-individual variation of VEGF concentrations, and the highest baseline and incremental levels were observed before HELP-apheresis (555 to 836 ng/ml) in the only study participant who had marked anginal pain during the stress studies.

DISCUSSION

Long-term transplant survival involves a number of adaptation processes in the hemodynamic environment to accommodate the allograft for daily physiologic needs, despite advanced graft arteriosclerosis. The present study has demonstrated that a 50% reduction of most of the relevant atherogenic blood compounds after a single HELP-apheresis treatment has significant hemodynamic implications for cardiac allograft macro- and microcirculation. Remarkably, these findings suggest that the vasomodulating effects occurred in the absence of nerve control of coronary vasomotion, because even partial reinnervation would have mainly affected the left anterior descending coronary artery (LAD), but we observed no relevant differences in the different vascular territories following apheresis.

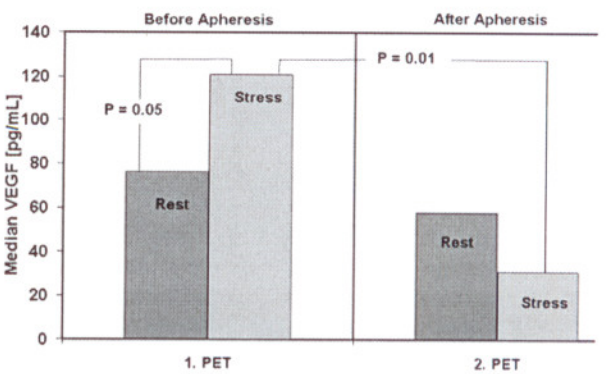

Figure 3. Increment of plasma VEGF concentrations during both rest/stress PET studies with a significantly lower increment during stress after apheresis, indicating better ischemic tolerance.

In contrast to HELP-apheresis/PET studies conducted earlier in native atherosclerosis,[7,12] we found a difference with regard to the relatively pronounced increase of resting flow after apheresis (+17.5%), which accounted for the insignificant increase in CFR. This may have been coincidental or related to the relatively small number of study participants. On the other hand, we observed generally lower arterial blood pressures the second day, that accounts for the higher corrected rest flow after apheresis which implies integration of the rate-pressure product. It is also possible that the rheologic effects of HELP-apheresis treatment played a greater role in the denervated heart than in native arteriosclerosis. A further possibility is that the lower arterial blood pressure on the second day of the study may indicate less patient stress, because they had become familiar with the PET procedure.

Basically, prevention of ischemia in the allograft is paramount to secure graft longevity. Because one distinctive feature of coronary circulation is the fact that oxygen extraction from blood is already maximal under resting conditions, with very little anaerobic reserve, increasing blood flow can best enhance oxygen supply. This applies equally to heart transplant patients. Unlike healthy subjects, oxygen extraction from blood appears to be lower and the venous oxygen content higher for heart transplant patients; consequently, long-term resting blood flow is higher, and the vessel becomes more dilated to match physiologic demands.[33] Blood flow, in turn, depends on driving pressure and coronary vascular resistance. The driving pressure depends on heart rate and arterial blood pressure, both of which are taken together in the "rate-pressure product," with the latter known to be nearly linearly coupled to oxygen consumption within wide flow ranges.[33]

In agreement with previous studies of heart transplant patients,[33,34] the rate-pressure product measured for our study patients tended to be higher than for healthy subjects. We found a decline after the apheresis that, although not reaching statistical significance ($p = 0.07$), could indicate less oxygen demand or improved oxygen delivery after apheresis. Theoretically, resting flow is largely independent of either humoral or nervous stimuli.[35] An increased resting flow may be the consequence of either an increased heart rate or changes in hemorheology. In the present study, the heart rate did not change, and the higher rest flow probably originated from the improved hemorheology.

Our findings, especially the increase in both rest and hyperemic flow, suggest a contribution of hemorheology. In contrast to the classical way to improve hemorheology—that is, hemodilution—HELP-apheresis has the advantage of maintaining the erythrocyte concentration (the hematocrit was stable), although it drastically lowers fibrinogen, plasma viscosity and erythrocyte aggregation. Given an appreciable reduction of the aforementioned blood compounds within the microcirculation, a facilitated oxygen delivery to the tissue is the logical consequence, with improved "ischemic tolerance."

An improved ischemic tolerance after apheresis corresponds well with some other observations: First, clinical reports from patients with coronary or peripheral artery disease have often described a relief of anginal pain and a longer pain-free walking distance, which was noted directly after HELP-apheresis and was maintained for several days.[29] Second, our measurements of plasma VEGF give additional evidence in favor of an improved ischemic tolerance after apheresis. Before apheresis, the plasma VEGF levels averaged 76 ng/ml with a positive increment of 60% during hyperemia, whereas after apheresis they were lower and declined during hyperemia (Figure 3). From the latter it can be deduced (a) that the release of VEGF might have been stimulated by nervous perception—as seen in the 1 patient with extremely high levels and signs of reinnervation (see Results); (b) VEGF release may be positively related to cholesterol, fibrinogen concentration or plasma viscosity and erythrocyte aggregation; (c) conversely, stress situations like hyperemia promote vascular deposition of high cholesterol, fibrin, CRP and lipoprotein (a), because stimulated VEGF levels used to increase vascular leakiness strongly; (d) finally, increased cholesterol, lipoprotein (a) and fibrinogen levels promote deposition of these molecules in the coronary arteries of CAV patients by raising VEGF levels.

In this study, the hyperemic flow after apheresis was 27% higher. Theoretically, such an increase could follow increases in arterial blood pressure, but the opposite was noted in the present study, suggesting that other mechanisms were operational.

Remaining control mechanisms can be nervous or metabolic in origin. With respect to *nerve control*, the effect of denervation with transplantation is to isolate the heart from anatomically mediated reflexes while enhancing its sensitivity to circulating catecholamines. Cardiac denervation can explain the resting tachycardia of the study participants due to the absence of vagal tone, and may cause the more gradual increase of heart rate with exercise or hyperemia, respectively (Figure 2). However, with time after transplantation, the likelihood of sympathetic but not parasympathetic reinnervation increases, and was shown to positively influence cardiac perfusion and the capacity to respond to stress situations.[34,36] Hence, these effects do not occur overnight, and they do not explain the difference before and after apheresis.

By contrast, pharmacologic α-adrenoreceptor blockade diminishes coronary vascular resistance by 25% in healthy subjects, but had no further effect in heart

transplant patients.[35] Other studies have indicated that the coronary flow reserve does not change in response to blockade of α- and β-adrenergic receptors,[35] and that the coronary flow reserve, as assessed by intracoronary application of papaverine, does not differ between innervated and denervated hearts.[35]

Focusing the impact of *metabolic control* on coronary perfusion, one must consider the complexity of adaptation processes after transplantation with mutually dependent immunologic, inflammatory, hemostatic and lipoprotein alterations. The participants in the present study belonged to a higher risk category with regard to their overall risk profile and mostly overt CAV. Considering the functional implications arising from a lowering of the aforementioned atherogenic blood compounds, we have noted the effects of *cholesterol reduction*, which showed a significant relief of vasotonus regardless of the method used, with LDL apheresis producing more rapid changes than statins or diet. Similarly designed studies from patients with isolated elevation of lipoprotein (a) have suggested effects on coronary perfusion similar to those described for LDL-cholesterol.[37] The effects of a selective lowering of the CRP on vasomotion appear to remain unclear.

The benefits arising from a rapid lowering of fibrinogen are very clear. We observed a baseline hyperfibrinogenemia (mean 4.65 ± 0.95 g/liter) that increased plasma viscosity by 10% and erythrocyte aggregation by 35% above normal. Patients were prone to atherothrombotic complications through impairment of microcirculatory flow, shear stress damage at the blood–endothelial interface, facilitation of plasma protein interactions with the endothelium in post-stenotic re-circulation areas, and increased propensity for thrombosis. Izumi et al[38] showed that using batroxibin for defibrination increases brain perfusion: specifically, a 68% fibrinogen reduction produced 10% higher CO_2 reactivity as an indicator of flow reserve.[38]

We compared the study participants under rest and hyperemia, because hyperemia allows for earlier disclosure of insufficient myocardial perfusion. Despite a decreased hyperemic capacity compared with healthy subjects we found rather comparable perfusion even in the patients with serious CAV. These findings are in line with other PET studies in heart transplant patients, supporting evidence for maintenance of a homogeneous global and regional perfusion.[33,34] Primary vasodilation is maintained in heart transplant patients through high nitric oxide levels,[39] a finding confirmed herein, although there was no further increase in response to adenosine. Aside from methodologic approach as a possible explanation for lack of sensitivity of nitrite/nitrate measurements, it is possible that the timing of the measurement (2 minutes after start of the adenosine infusion) was not adequate, or the basal activity was already maximal, allowing no further stimulation. The latter explanation would correspond well with the weak increase in coronary flow reserve that we observed.

In looking for other possibly relevant mediators we could exclude the vasodilator, BNP, which was, on average, 16% lower after apheresis (comparison of median), and decreased further with long-term application of apheresis. We can also exclude endothelin, which is known to increase in concentration after apheresis.[6]

In conclusion, the present findings provide evidence for a functional effect of HELP-apheresis for improving myocardial macro- and micro-perfusion, and probably also ischemic tolerance, in heart transplant patients. Our findings complement and expand the general understanding of the outcome data on this issue. Finally, all study participants were already on long-term HELP-apheresis therapy, which could be expected to maximize the normalization of their vasoreactivity and minimize subsequent changes with each treatment. Given the severity of CAV in most patients, we conclude that regular apheresis treatment helps to sustain a mostly homogeneous perfusion under rest and stress conditions and is capable of meeting the metabolic needs of patients.

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