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ORIGINAL ARTICLE

Vascular resistance and endothelial function in cyclosporine-treated lung transplant recipients

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Summary

The majority of patients undergoing solid organ transplantation develop hypertension, to which vasoconstriction and impaired endothelial function have been suggested to contribute. We compared basal vascular resistance and nitric oxide-mediated endothelial-dependent and independent vasoreactivity between cyclosporine-treated lung transplant recipients and healthy subjects. Forearm blood flow was measured by venous occlusion plethysmography at rest and during acetylcholine, glyceryltrinitrate and N(G)-monomethyl-L-arginine acetate (L-NMMA) infusion in 11 lung transplant recipients 3–5 years after transplantation and in eight healthy subjects. Forearm vascular resistance (FVR) was calculated. Plasma levels of endothelin-1 (ET-1) and von Willebrand factor (vWf) were analysed. Basal vascular resistance was 40% lower in transplant recipients than in healthy subjects ($P = 0.021$). Endothelial-dependent and independent vasodilation did not differ. Plasma levels of ET-1 and vWf were higher in transplant recipients ($P = 0.009$ and $P < 0.001$ respectively). There was a significant correlation between ET-1 levels and FVR in healthy subjects ($r = 0.83$, $P = 0.042$), but not in transplant recipients ($r = -0.14$, $P = 0.70$). The findings oppose the theory of generalized vasoconstriction and impaired endothelial function in the pathogenesis of hypertension after transplantation. Increased plasma levels of ET-1 do not cause increased FVR in lung transplant recipients.

Introduction

The majority of patients undergoing solid organ transplantation develop hypertension [1–5], which contributes to cardiovascular morbidity and mortality after transplantation [4,6]. The exact mechanisms behind post-transplant hypertension are not fully understood but it is evident that immunosuppressive drugs, such as cyclosporine A (CsA) and corticosteroids play an important role [4,6]. CsA may contribute to the development of post-transplant hypertension through different mechanisms. These include direct vasoconstriction, stimulation of vasoconstrictor hormones and reduced activity of endothelial-dependent vasodilator pathways [4,6]. Accordingly, increased systemic vascular resistance due to CsA-induced vasoconstriction has been suggested to be responsible, at least partially, for the development of post-transplant hypertension [7,8].

It is unclear if all vascular beds contribute to the increased systemic vascular resistance. Our group and others have presented data that questions the presence of increased skeletal muscle vascular resistance in CsA treated lung and heart transplant recipients [9–11]. However, our investigations were performed relatively early after transplantation (1–18 month) in lung recipients without manifest hypertension. It is possible that general peripheral vasoconstriction occur at a later stage after transplantation, when the majority of recipients have developed hypertension.

In this study, we hypothesized that increased forearm vascular resistance (FVR) is detectable in lung transplant

recipients late after transplantation and is associated with impaired endothelial function. Accordingly, we investigated basal vascular resistance in CsA treated lung transplant recipients late (36–48 month) after transplantation. Furthermore, we sought to assess forearm endothelial function by means of endothelial-dependent vasodilatation and by measuring plasma levels of endothelin-1 (ET-1) and von Willebrand factor (vWf). Finally, we investigated endothelial-independent nitric oxide (NO)-mediated vasodilatation and the effect of endogenous NO blockade on forearm blood flow (FBF).

Patients and methods

Patients

Eleven transplant recipients (mean time after transplantation 40 ± 2 month, range 36–48) and eight age- and gender-matched healthy subjects were included in the study. Baseline characteristics are given in Table 1. Five of the recipients had single lung transplantations; five had double lung transplantations and one heart–lung transplantation. The pretransplant diagnoses were emphysema ($n = 3$), primary pulmonary hypertension ($n = 4$), idiopathic pulmonary fibrosis ($n = 1$), cystic fibrosis ($n = 1$), Eisenmenger syndrome ($n = 1$) and Histiocytosis X ($n = 1$). All recipients were on triple immunosuppression with cyclosporine, azathioprin ($n = 7$) or mycophenolate mofetil ($n = 4$) and prednisone. Mean cyclosporine dose was 3.2 ± 0.3 mg/kg/day and mean blood cyclosporine trough level 161 ± 10 μg/l. Mean Cr-clearance in the recipients was 37 ± 4 ml/min (range 10–53).

Nine of the 11 transplant recipients had developed post-transplant hypertension and were treated with one or more antihypertensive drugs (beta-blockers $n = 5$, calcium channel blockers $n = 5$ or angiotensin II blockers $n = 6$). Actual antihypertensive medication is given in Table 2. All antihypertensive medication was discontinued 24 h before examination: None of the transplant recipients had any ongoing infection or rejection. The Research Ethics Committee of the Medical Faculty, University of Göteborg, approved the study protocol. All participants gave informed consent.

None of the participants in the study were treated with nonsteroidal anti-inflammatory drugs. Four of the recipients were treated with statins. All subjects abstained from caffeine-containing drinks for 12 h before any measurements were performed. All participants were nondiabetic and nonsmokers. All studies were performed in a quiet room maintained at a controlled temperature between 22 and 24 °C.

Measurements

Subjects rested throughout the study with both forearms at the level of the heart. The brachial artery of the non-dominant arm was cannulated with a 20 Gauge cannula under lidocaine local anaesthesia (Xylocain 1% Astra Pharmacueticals Ltd, Gothenburg, Sweden). Drugs were dissolved in physiological saline (0.9%, B Braun Medical Ltd, Bromma, Sweden) and prepared aseptically from sterile stock solutions on the day of the study. The infusion rate was kept constant at 100 ml/h. Before administration of drugs, saline was infused for at least 30 min followed by baseline measurements.

Forearm blood flow was measured simultaneously in both arms by venous occlusion plethysmography using mercury in silastic strain gauges applied to the widest part of the forearm [12]. To exclude the circulation in the hands, wrist cuffs were inflated to 200 mmHg 1 min prior to and during each measurement. Upper arm cuffs were inflated intermittently to 40 mmHg in order to temporarily prevent venous outflow from the forearm and thus obtain plethysmographic recordings.

Table 1. Baseline characteristics.

	Transplant recipients	Healthy subjects	*P*-value
n	11	8	
Mean age (years)	47.8 ± 4.4	47.6 ± 2.2	0.23
Male/Female	6/5	4/4	0.84
Basal blood pressure (mmHg)			
Systolic	147 ± 6	129 ± 5	0.034
Diastolic	74 ± 3	71 ± 3	0.40
Mean	102 ± 4	94 ± 3	0.14
Pulse pressure	73 ± 6	59 ± 3	0.083
Basal heart rate/min	73 ± 3	62 ± 3	0.10
Basal forearm blood flow (ml/100 ml tissue/min)	5.0 ± 0.7	2.6 ± 0.4	0.027
Basal forearm arterial resistance (mmHg/ml/100 ml tissue/min)	25.4 ± 4.7	42.1 ± 6.9	0.021

Mean ± SEM or number.

Table 2. Anti-hypertensive medication in 11 lung transplant recipients. All medication was discontinued 24 h before the investigation.

Drug	Number of patients
Ca-channel blocker	2
Ca-channel blocker + β-blocker	1
Ca-channel blocker + AT-II-antagonist	1
Ca-channel blocker + β-blocker + AT-II-antagonist	1
AT-II-antagonist	1
AT-II-antagonist + β-blocker	3
None	2

AT-II, angiotensin II.

A dual channel strain gauge plethysmograph (Elektromedicin AB, Kullavik, Sweden) was used and calibrated before each measurement. Absolute blood flow in both forearms was obtained from the mean of at least three consecutive measurements for each measurement period. The ratio of flows in the infused and noninfused arms was calculated and expressed as percentage change from baseline. FVR was calculated as mean arterial pressure (MAP)/FBF (mmHg/ml/100 ml tissue/min). Heart rate (HR) and arterial pressure (systolic, diastolic and mean) were monitored on a Sirecust 1281 screen (Siemens Medical Electronics Inc, Danvers, MA, USA) in the nondominant arm immediately after each blood flow measurement.

After baseline infusion of saline and baseline measurements, the endothelial dependent vasodilator acetylcholin (Miochol®, Novartis Ophthalmics, Täby, Sweden) was infused in increasing doses (10, 30 and 60 μg/min). Each step was maintained for 5 min after which FBF and MAP were measured. After a 30 min washout period, and new baseline measurements, the exogenous NO donor glyceryltrinitrate (GTN) was infused (Tika Läkemedel, Lund, Sweden) in increasing doses (0.05, 1 and 10 nmol/min). Each step was maintained for 5 min after which FBF and MAP were measured. After another 30-min washout period and baseline measurement, the nitric oxide synthase inhibitor N(G)-monomethyl-L-arginine acetate (L-NMMA) (Clinalfa, Darmstadt, Germany) was infused for 5 min at 4 μmol/min followed by FBF and MAP measurements.

Laboratory analyses

Blood was drawn from the arterial cannula and collected into EDTA (ET-1) or citrate (vWF) tubes after the first baseline measurement. Samples were put on ice until centrifuged at 4 °C for 20 min (2000 ***g***) within 15 min. Plasma samples were stored at −70 °C until analysis. Plasma concentrations of ET-1 were analysed with radioimmunoassay (RPA545, Amersham Bioscience, Uppsala, Sweden), and vWF-antigen levels were determined with an immunoenzymatic method (DakoCytomation Norden AB, Älvsjö, Sweden).

Statistics

Continuous data were compared between the groups with the nonparametric Mann–Whitney test and correlations were calculated with Spearman test. Categorical data were analysed with chi-square test. FBF, FVR, MAP and HR comparisons between transplant recipients and controls during Ach, GTN and L-NMMA infusion were performed with ANOVA for repeated measurements. Changes from baseline within groups were evaluated with Wilcoxon's paired test. All results are expressed as mean ± standard error of the mean (SEM). A P-value of <0.05 was considered statistically significant.

Results

Resting blood pressure and heart rate

Resting systolic blood pressure was, and HR tended to be, higher in the transplant recipient group, Table 1. There were no significant alterations in MAP or HR during infusion of Ach, GTN or L-NMMA in any group.

Resting forearm blood flow and regional vascular resistance

Baseline FBF was higher and FVR lower in the transplant group compared with controls (FBF 5.0 ± 0.7 vs. 2.6 ± 0.4 ml/100 ml tissue/min, $P = 0.027$, FVR 25 ± 4.7 vs. 42 ± 6.9 mmHg/ml/100 ml tissue/min, $P = 0.021$), Table 1.

Endothelial-dependent vasodilatation

Forearm vascular resistance increased and FVR decreased significantly in both groups during Ach infusion, Fig. 1 and Table 3. The changes in FBF and FVR tended to be more pronounced in the transplant group but the differences did not reach statistical significance ($P = 0.15$ and $P = 0.08$ respectively).

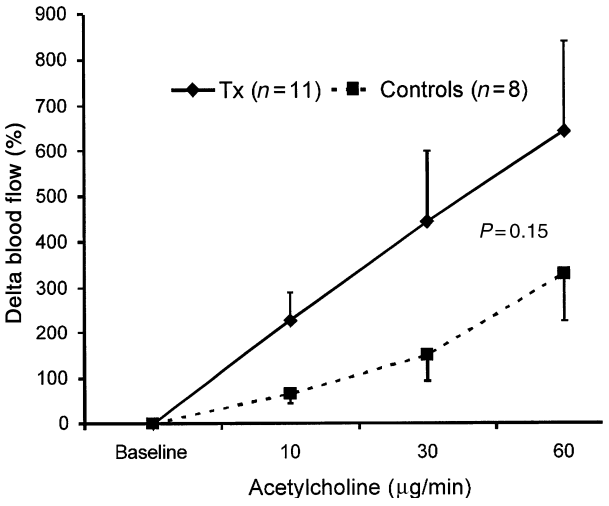

Figure 1 Relative changes in forearm blood flow after infusion of acetylcholine in healthy subjects and in transplant recipients. Tx, lung transplant recipients.

Table 3. Relative changes in forearm blood flow and forearm vascular resistance during Ach, GTN and l-NMMA infusion in transplant recipients ($n = 11$) and healthy subjects ($n = 8$).

	Relative blood flow (%)		Relative vascular resistance (%)	
	TPX	CTRL	TPX	CTRL
Ach (μg/min)				
0	0	0	0	0
10	227 ± 62**	65 ± 21*	−59 ± 7**	−30 ± 11*
30	444 ± 154**	149 ± 56*	−68 ± 8**	−46 ± 10*
60	642 ± 198**	330 ± 105*	−71 ± 8**	−60 ± 13*
GTN (nmol/min)				
0	0	0	0	0
0.05	−16 ± 7	8 ± 12	18 ± 19	4 ± 16
1.0	45 ± 13*	30 ± 14	−25 ± 8*	−15 ± 11
10.0	197 ± 44**	139 ± 42*	−60 ± 5**	−47 ± 12*
l-NMMA (μmol/min)				
0	0	0	0	0
4	−25 ± 14	−10 ± 8	69 ± 27*	16 ± 11

Values are mean ± SEM.
Ach, Acetylcholine; GTN, glyceryl trinitrate; l-NMMA, N(G)-monomethyl-l-arginine acetate; TPX, transplant recipients; CTRL, healthy subjects.
*$P < 0.05$ vs. baseline; **$P < 0.01$ vs. baseline.

Endothelial-independent vasodilatation

Forearm vascular resistance increased and FVR decreased in both groups during GTN infusion without significant intergroup differences, Figure 2 and Table 3.

Nitric oxide synthase inhibition

Compared with baseline, infusion of L-NMMA significantly increased FVR (+69% ± 27%, $P = 0.022$) and tended to decrease FBF (−25% ± 14%, $P = 0.09$) in transplant recipients. In control subjects, the effect was not statistically significant (+16 %± 11%, $P = 0.11$ and −10 ± 8, $P = 0.21$, respectively). The intergroup differences were not statistically significant ($P = 0.12$ and 0.40 respectively).

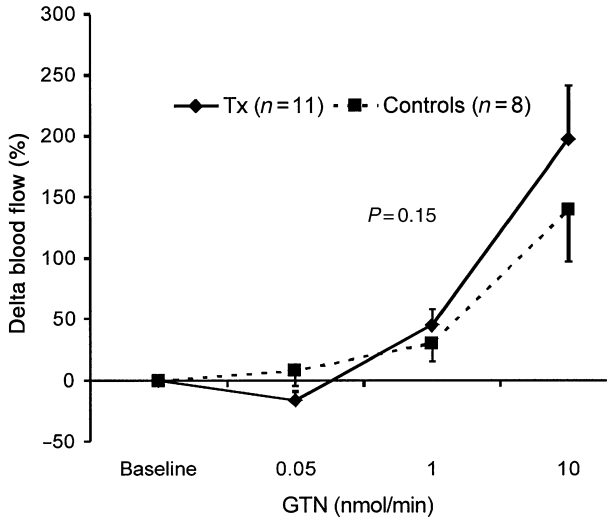

Figure 2 Relative changes in forearm blood flow after infusion of glyceryl trinitrate in healthy subjects and in transplant recipients. Tx, lung transplant recipients.

Endothelin-1 and von Willebrand factor

Baseline plasma levels of ET-1 (21.9 ± 1.7 vs. 15.0 ± 1.6 pg/ml, $P = 0.009$) and vWf (1.7 ± 0.2 vs. 0.7 ± 0.1 IE/ml, $P < 0.001$) were higher in the transplant recipients compared with the control group. There was a significant correlation between ET-1 and vWF levels ($r = 0.49$, $P = 0.046$).

In healthy subjects, there were significant correlations between basal ET-1 levels and basal FBF and FVR ($r = −0.94$, $P = 0.005$,) and $r = 0.83$, $P = 0.042$, respectively), Fig. 3a. These correlations were not present in transplant recipients ($r = −0.30$, $P = 0.39$ and $r = −0.14$, $P = 0.70$), Fig. 3b. There were no significant correlations between ET-1 and vWf and endothelial dependent vasodilatation ($P = 0.35$ and 0.43, respectively), and no significant correlations between dose and concentration of CsA and plasma levels of ET-1, vWf or endothelial dependent vasodilatation.

Discussion

The main findings in the present study were that, no evidence for increased vascular resistance or impaired endothelial-dependent responses to vasoactive substances

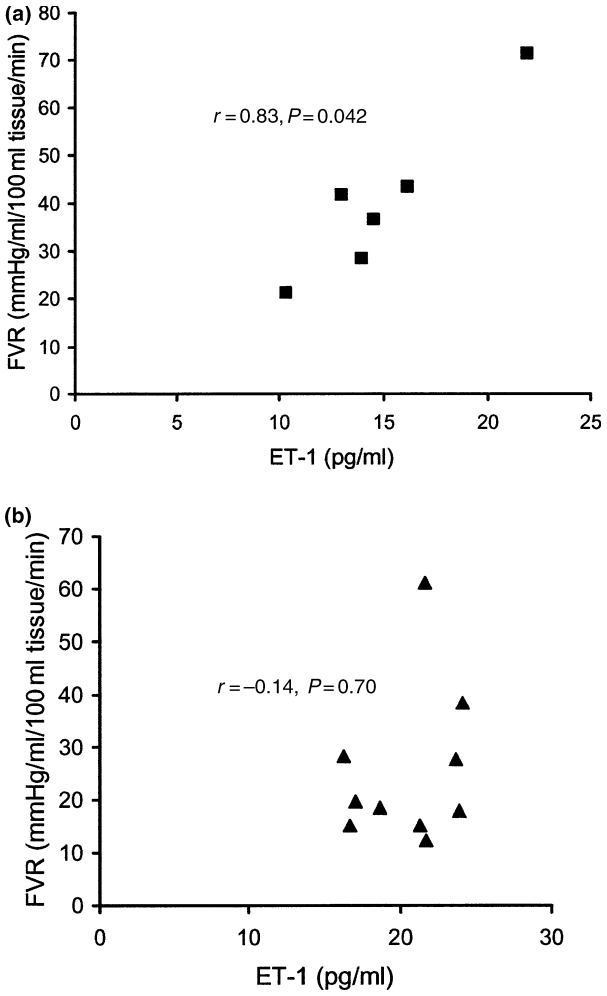

Figure 3 Correlations between basal plasma levels of endothelin-1(ET-1) and basal forearm vascular resistance in healthy subjects (a) and in transplant recipients (b).

could be detected in lung transplant recipients after long-term treatment with cyclosporine. The findings argue against generalized vasoconstriction and impaired endothelial function as a major cause of transplant-associated hypertension.

Post-transplant hypertension and vascular resistance

The prevalence of hypertension in transplant recipients is markedly higher than in nontransplant populations [5,6]. The mechanisms behind post-transplant hypertension are incompletely understood but it is evident that treatment with CsA is one important factor [4]. CsA have significant vascular effects that may promote hypertension. In experimental studies, CsA induces direct smooth muscle cell contractions [13,14] and increases production of ET-1, a potent vasoconstrictor [15,16]. Furthermore, CsA has been suggested to impair endothelial function, resulting in decreased production of endothelial-derived NO [17,18]. Thus, increased systemic vascular resistance due to CsA-induced vasoconstriction and endothelial dysfunction has been suggested to contribute to post-transplant hypertension [8]. Whether this is a generalized effect in all vascular beds has previously been questioned by Bracht *et al.* [11], who found lower FVR despite higher blood pressure in heart recipients late after transplantation. In addition, in two recent studies, we found no evidence of increased FVR in lung transplant recipients [9,10]. However, those latter investigations were performed in lung recipients within 18 month after transplantation and without hypertension, and it is possible that vasoconstriction and increased FVR occur later. Therefore, the present investigation was designed to investigate FVR and vascular function 36–48 month after lung transplantation. Again, we found no evidence of increased resting vascular resistance in the forearm. On the contrary, transplant recipients had 40% lower basal vascular resistance than matched healthy subjects, Table 1. Thus, the present results question again the concept of generalized vasoconstriction as a major factor for hypertension in cyclosporine-treated lung transplant recipients. However, transplant recipients have higher blood pressure than healthy subjects and as MAP is the product of cardiac output and systemic vascular resistance other explanations need to be explored. Increased vascular resistance in other vascular beds, such as the renal vasculture may contribute, as suggested by van den Dorpel *et al.* [19] in renal transplant recipients. If pronounced vasoconstriction is present in other vascular beds, the decreased FVR in transplant recipients could be a secondary event because of compensatory skeleton muscle vasodilatation. At first sight such a relaxation is not consistent with the increased plasma levels of the potent vasoconstrictor ET-1 in the brachial artery. However, we found a correlation between FVR and ET-1 only in healthy subjects, not in transplant recipients. This suggests that there is an impaired forearm vasoconstrictive response to ET-1 in hypertensive lung recipients, possibly on receptor levels. This hypothesis is supported by previous studies in transplant recipients demonstrating impaired forearm vasodilatation after selective ET-a receptor blockade [9,20].

Nitric oxide pathway

Endothelial-dependent vasodilatation

In the present study, we could not detect any signs of impaired endothelial-dependent vasorelaxation in the forearm during infusion of Ach in lung transplant recipients compared with healthy subjects. In fact, there was a tendency towards an enhanced response to Ach in trans-

plant recipients compared with the matched healthy subjects, Figure 1 and Table 3. This absence of impairment in endothelial-dependent vasorelaxation is in conflict with previous investigations in transplant recipients. Holm *et al.* [21] found impaired endothelial-dependent vasodilatation with skin laser-doppler in heart transplant recipients and Passauer *et al.* [20] found impaired response to Ach in renal transplant recipients investigated by venous occlusion plethysmography. However, Cifkova *et al.* [22] could not detect any signs of impaired endothelial-dependent vasodilatation despite elevated blood pressure in a longitudinal study before and after liver transplantation. The diverging results in the different studies may thus be explained by higher incidence of pretransplant vascular dysfunction in heart and renal transplant recipients than in lung and liver recipients [2,5,23].

Endothelial-independent vasodilatation

Endothelial-independent vascular function was investigated by means of change in FBF during infusion of the NO donor GTN. We could not detect any difference in endothelial-dependent dilation between transplant recipients and controls, which is accordance with our investigation early after lung transplantation [10]. Preserved response to NO-donating substances has also been demonstrated after liver [24] and renal transplantation [25]. Thus, it appears that transplantation and long-term treatment with CsA do not influence smooth muscle sensitivity to NO.

Nitric oxide-synthase inhibition

In healthy subjects, endothelial production of NO is crucial for maintaining normal basal vascular tone. Whether CsA increases or decreases endogenous NO production is a matter of discussion. In experimental studies, CsA reduces NO production [17,18], while one human study has shown the opposite [26]. In the present study, we found a significant rise in relative FVR in the transplant recipients when endogenous NO-production was inhibited by L-NMMA, despite the lower basal FVR compared with healthy subjects. This indicates that the decrease in resting FVR in hypertensive lung transplant recipients 3–4 years after transplantation is dependent on endogenous NO-production. This is in accordance with the discussion above, about a possible NO-mediated compensatory vasorelaxation in forearm vasculature in hypertensive lung transplant recipients.

Endothelin-1

Increased plasma concentrations of the potent endogenous vasoconstrictor ET-1 was demonstrated in CsA treated lung transplant recipients, which is in accordance with previous experimental studies [16,27] and in clinical investigations in heart [28] and kidney transplant recipients [29]. ET-1 is secreted from endothelial cells upon different stimuli such as CsA [27] and tacrolimus [30]. In the present study, the ET-1 levels were similar to the ones we previously found in transplant recipients early after transplantation, using the same methodology [10]. This suggests that the elevation of plasma ET-1 is initiated early after transplantation and persists over time. In the present study, the increased arterial plasma level of ET-1 was not associated with forearm vasoconstriction. This may be due to down-regulation of ET-A receptors in the skeletal muscle vessels, as discussed above. The results do not exclude that ET-1-mediated vasoconstriction may be present in other vascular beds causing a rise in systemic vascular resistance, and it has been shown that CsA induces renal vasoconstriction mediated by ET-1 [31]. Other characteristics of ET-1 may also be of importance in the development of post-transplant hypertension. ET-1 has proliferative properties on smooth muscle cells [32] and increased arterial stiffness has been demonstrated after lung [10] and renal transplantation [25].

von Willebrand factor

von Willebrand factor is a glycopeptide which is essential for platelet adhesion to the endothelium [33]. Plasma levels of vWf have been suggested to reflect endothelial activity and function [34] and are increased in patients with congestive heart failure and ischemic heart disease [33,34]. In addition, increased levels have been reported in heart transplant recipients [33,35] and there is also a report of an association between cyclosporine and vWf [35].

In the present study, we found increased plasma levels of vWf but no evidence of impaired endothelial-dependent vasorelaxation. In addition, there were no correlations between vWf levels and grade of endothelial-dependent vasodilatation or dose or concentration of CsA. Taken together, these finding suggest that vWf and Ach infusion measures different aspects of endothelial function or activity and thus cannot be used interchangeable to assess endothelial function.

Study limitations

Even if measuring response to vasoactive agents by strain-gauge plethysmography is considered to be the gold standard in assessing endothelial function in resistance arteries [36], it should be pointed out that this method also has limitations. For instance, interpretation and comparing results between groups may be obscured if initial

resting blood flow or blood pressure differ between the groups [12]. On the other hand, no other method has been proven to be more accurate [36]. Further, limitations are the variability of the included lung recipients and that, anti-hypertensive drugs were discontinued first 24 h before the examinations, Table 2. We cannot exclude that active substances may still be present in plasma at this time point. However, discontinuation of medication for a longer period of time was judged unethical.

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