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ORIGINAL ARTICLE

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The effect of cyclosporin A on the level of big endothelin in patients one year after orthotopic heart transplantation

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Abstract Orthotopic heart transplantation (OHTx) is currently an established method for the treatment of end-stage heart failure. Persistent elevated plasma endothelin-1 (ET-1) levels have been reported after successful OHTx, the etiology of which is not yet fully understood. Immunosuppressive therapy is assumed to be one of the possible factors affecting ET-1 levels in the body. The present study evaluated the effect of cyclosporin A (CyA) on big ET-1 levels (a precursor of ET-1) in patients 1 year after successful OHTx. The study population comprised 34 patients after OHTx (28 males, 6 females, mean age 49.56 ± 11.83 years) divided into two groups according to immunosuppressive protocol (17 patients on cyclosporine–azathioprine–prednisone and 17 patients on cyclosporine-mycophenolate mofetil-prednisone therapy). Plasma levels of big ET-1 and CyA were available for all patients. The control groups consisted of 10 healthy individuals (8 males, 2 females, mean age 41.1 ± 11.55 years) and 20 patients with severe heart failure (15 males, 5 females, mean age 54.45 ± 8.49 years), respectively. Big ET-1 plasma levels were found to be elevated in OHTx patients in comparison with healthy controls (13.63 ± 11.3 fmol/ml vs 2.99 ± 1.98 fmol/ml, $P = 0.005$). Big ET-1 plasma levels correlated with plasma CyA levels in patients treated with cyclosporine–azathioprine–prednisone ($r = 0.53$, $P = 0.03$). This was not the case in either in the OHTx patients as a whole or in the subgroup of patients on cyclosporine–mycophenolate mofetil–prednisone therapy. The plasma levels of big ET-1 are dependent on CyA plasma levels 1 year after successful OHTx in patients treated with the immunosuppressive combination of cyclosporine, azathioprine, and prednisone. As this finding was not observed in the mycophenolate group of patients, mycophenolate mofetil might affect the alteration of the endothelin metabolism.

Keywords Orthotopic heart transplantation · Big endothelin-1 · Cyclosporine

Introduction

Orthotopic heart transplantation (OHTx) is an established method for the treatment of end-stage heart failure. Elevated levels of some neurohormones—inter alia endothelin-1 (ET-1) and big ET-1—are found in patients with advanced heart failure. These constitute part of the syndrome of neurohormonal system activation associated with heart failure and responsible for its progressive nature. The levels of ET-1 and big ET-1

correlate with the prognosis of patients with advanced heart failure [1]. Since successful OHTx is followed by substantially improved hemodynamics, normalization of neurohumoral activity could be expected. However, persisting elevated plasma ET-1 levels are a typical finding after OHTx [2, 3, 4, 5], but the cause of the increase is not yet fully understood. Increased ET-1 levels have also been reported after transplantation of other organs or tissues, e.g., the liver [6] and bone marrow [7]. A factor that possibly causes alterations of ET-1 levels after transplantation may be the immunosuppressive drugs used, and cyclosporine A (CyA) in particular. CyA enhances the synthesis of ET precursors by both rat [8] and human endothelial cells [9, 10] in vitro, and the same observations were made in experiments with mice [11, 12]. Elevated levels of ET-1 were also observed in non-transplant patients receiving CyA for idiopathic uveitis [13].

Big ET-1 is a precursor of ET-1 with very low biological activity. It is cleared from circulation more slowly than ET-1. Therefore, big ET-1 circulates in higher concentrations in the case of ET-1 overproduction. Because of its characteristics, the current literature assumes big ET-1 to be more suitable for evaluating endothelin production in the organism than ET-1 alone [1].

To the best of our knowledge, the effect of CyA on the systemic levels of big ET-1 following clinical OHTx has not been assessed to date. The present study was therefore designed to evaluate the relationship of plasmatic big ET-1 levels to serum CyA levels in patients 1 year after successful OHTx. Control groups included patients with advanced heart failure and healthy controls.

Patients and methods

Patients

Of 70 patients after OHTx performed between February 1999 and October 2000, 34 subjects with available data on plasma big ET-1 levels at 1 year after the procedure were included in the study. All patients were rejection-free (Banff: <2) and had good graft function (normal systolic function upon echocardiographic examination, i.e., left ventricular ejection fraction $>50\%$) at the time of blood sample collection (368 ± 14 days after OHTx). The control groups comprised both 20 patients with advanced chronic heart failure (EF $\leq 20\%$, NYHA Class III–IV) and 10 healthy controls. The characteristics of these groups are shown in Table 1. The groups did not differ in their basic characteristics. To assess the influence of immunosuppressive therapy, and especially CyA, on plasma big ET-1 levels, the group of patients after OHTx was divided into two subgroups with different immunosuppressive protocols, one with CyA-azathioprine-prednisone (CyA-Aza-Pred, target CyA plasma levels: 300–400 ng/ml spec, Aza dosage: 1 mg/kg per day, prednisone: 0.09 ± 0.01 mg/kg per day) and the other with CyA-mycophenolate mofetil-prednisone (CyA-MMF-Pred, target CyA plasma levels: 200–300 ng/ml spec, MMF dosage: 0.03 g/kg per day, prednisone: 0.09 ± 0.01 mg/kg per day). These subgroups did not differ in their basic characteristics (Table 2). The protocol of the study was approved by the local ethics committee, and all patients signed their informed consent to inclusion in the study.

Methods

Blood collection for determination of plasma levels of big ET-1 was performed during hospitalization, in a fasting state, after a minimum bed rest of 30 min, with blood drawn from a peripheral vein. Patients after OHTx had the blood sample for CyA determination drawn at the same time (i.e., before the morning CyA dose).

CyA levels were determined by radioimmunoassay, specifically without metabolites (Immunotech Kit, Cat. No. 3440, Prague, Czech Republic). Big ET-1 levels were determined after non-ethanol extraction by radioimmunoassay (Phoenix Pharmaceuticals, Belmont, Calif., Cat. Code RK 023–10).

We tested the hypothesis that plasma big ET-1 levels remain elevated 1 year after OHTx and that big ET-1 levels are dependent on plasma CyA levels in OHTx recipients. All data are expressed as mean $\pm$ standard deviation (SD). The differences between the individual groups were evaluated using the t-test or Mann-Whitney test (for comparison of two groups) and the Kruskal-Wallis test (for comparison of three groups) for continued variables and the χ^2-test (with Yates' correction when necessary) for categorical variables. Linear regression analysis was used to examine the correlation between CyA and big ET-1 levels. P-values of less than 0.05 were considered to be statistically significant.

Results

The plasma levels of big ET-1 were found to be increased in the group of patients 1 year after OHTx compared to healthy controls (13.63 ± 11.3 fmol/ml vs 2.99 ± 1.98 fmol/ml, $P < 0.005$). No statistically significant difference in plasma big ET-1 levels was found between the groups of patients after OHTx and patients with advanced heart failure, although there was a trend

Table 1 Basic characteristics of study groups (*CAD* coronary artery disease, *CHF* chronic heart failure, *M/F* male/female, *OHTx* orthotopic heart transplantation)

*OHTx group vs group of CHF patients,
**OHTx group vs healthy controls

	OHTx	CHF	Healthy controls	*P*-value
Number of patients	34	20	10	
Sex: M/F	28/6	15/5	8/2	0.81
Mean age	49.56 ± 11.83	54.45 ± 8.49	41.1 ± 11.55	0.11*, 0.06**
Pre-Tx diagnosis:				
– Cardiomyopathy	18			
– CAD	11			
– Other diagnoses	5			

Table 2 Basic characteristics of subgroups with different immunosuppressive regimens (*CAD* coronary artery disease, *M/F* male/female, *CyA + MMF + Pred* cyclosporin A + mycophenolate mofetil + prednisone, *CyA + Aza + Pred* cyclosporin A + azathioprine + prednisone)

	CyA + MMF + Pred	CyA + Aza + Pred	*P*-value
Number of patients	17	17	
Sex: M/F	16/1	12/5	0.17
Mean age (years)	50.7 ± 11.35	48.41 ± 12.53	0.57
Pre-Tx diagnosis:			0.58
– Cardiomyopathy	8	10	
– CAD	6	5	
– Other diagnoses	3	2	
Diabetes mellitus	2	7	0.12
Body mass index	27.21 ± 4.35	24.97 ± 3.93	0.12
Cholesterolemia (mmol/l)	5.55 ± 1.25	5.39 ± 1.05	0.68
Creatininemia (μmol/l)	124.67 ± 31	128.83 ± 46.2	0.76

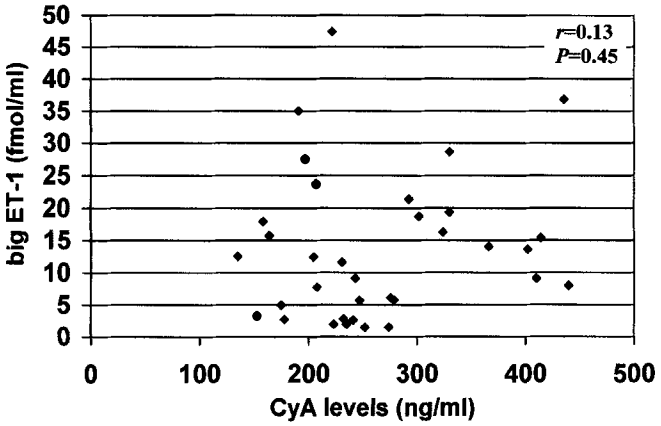

Fig. 1 Association between plasma cyclosporin A (*CyA*) and big endothelin-1 (*ET-1*) levels in patients after orthotopic heart transplantation

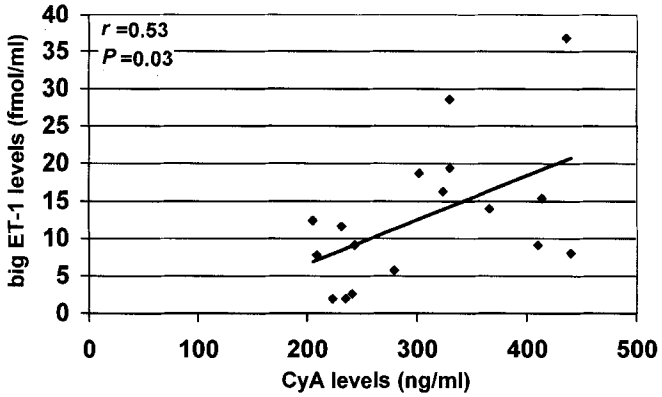

Fig. 2 Association between plasma cyclosporin A (*CyA*) and big endothelin-1 (*ET-1*) levels in the group of patients on the immunosuppressive regimen consisting of cyclosporin A–azathioprine–prednisone

towards increased big ET-1 levels in post-OHTx patients (13.63 ± 11.3 fmol/ml vs 8.19 ± 4.15 fmol/ml, $P = 0.06$). No difference in plasma big ET-1 levels was found between the subgroups of post-OHTx patients treated with the different immunosuppressive protocols (CyA-Aza-Pred vs CyA-MMF-Pred: 12.91 ± 9.32 fmol/ml vs 14.34 ± 13.23 fmol/ml, $P = 0.71$) in spite of different plasma CyA levels (307.47 ± 81.91 ng/ml vs 221 ± 86.26 ng/ml, $P = 0.001$).

No correlation between plasma CyA and big ET-1 levels was found in the group of patients 1 year after OHTx as a whole, regardless of the immunosuppressive protocol used ($r = 0.13$, $P = 0.45$, Fig. 1). The relationship between plasma CyA and big ET-1 levels in the group of patients 1 year after OTHx treated with the immunosuppressive regimen consisting of CyA-Aza-Pred is shown in Fig. 2. This relationship was found to be statistically significant ($r = 0.53$, $P = 0.03$). A similar finding was not made in the group of patients 1 year after OHTx treated with the immunosuppressive regimen consisting of CyA-MMF-Pred ($r = 0.07$, $P = 0.78$, Fig. 3).

Discussion

To the best of our knowledge this is the first work to study plasma big ET-1 levels in a population of patients after OHTx. The present paper has shown that plasma big ET-1 levels are elevated in patients 1 year after OHTx (368 ± 14 days in our case). A surprising finding is the degree of increase in big ET-1 levels, which tends to be even higher than in patients with advanced heart failure (EF ≤ 20%, NYHA Class III–IV). More importantly, this is the first study to demonstrate that plasma big ET-1 levels correlate with plasma CyA levels in patients treated with an immunosuppressive regimen consisting of CyA-AzA-Pred.

The origin of elevated ET-1 levels is still regarded questionable. Increased tissue ET-1 immunoreactivity was found within the graft endocardium, myocardium, and vessels affected by graft vasculopathy [14] in an animal model of heart transplantation, and Ravelli et al. reported on increased tissue ET-1 immunoreactivity in vessels affected by graft vasculopathy in humans [15]. Despite these findings, and considering the results of a

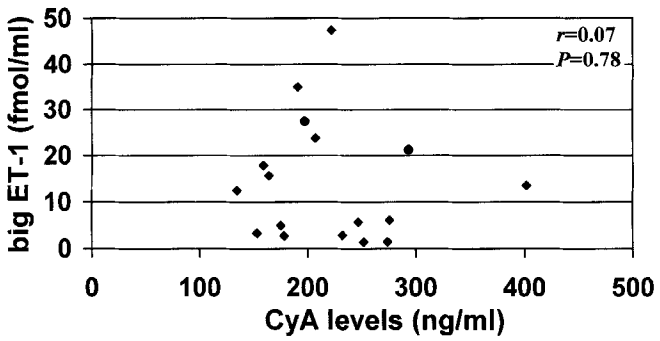

Fig. 3 Association between plasma cyclosporin A (*CyA*) and big endothelin-1 (*ET-1*) levels in the group of patients on the immunosuppressive regimen consisting of cyclosporin A–mycophenolate mofetil–prednisone

study by Weiss et al. [5], it can be assumed that the main cause of rising ET-1 levels after OTHx is extracardiac. According to Weiss et al. [5], even ET-1 uptake by the cardiac graft can be observed at elevated systemic levels. Taking the above into account as well as observations made with patients undergoing transplantation of other organs—the liver [6] and bone marrow [7]—the origin of increase in plasmatic levels of ET-1 can be assumed to be independent of the transplant organ. The factors common to all the above cases are (1) the presence of a chronic immune response to the antigen donor/recipient mismatch, (2) the possible presence of "chronic rejection", and (3) the presence of immunosuppressive medication. Despite the conclusions of animals studies [12] and the findings of Ravelli et al. [15], no association has been demonstrated to date between the plasmatic levels of ET-1 and morphological signs of graft vasculopathy in humans. Only according to Weiss et al. do changes in myocardial clearance of ET-1 (i.e., increase in cardiac ET-1 production and decrease in cardiac ET-1 uptake) correlate with microvascular and epicardial endothelial-dependent vasodilatation (to acetylcholine), but, again, these findings do not correlate with the morphological finding of graft vasculopathy [5].

CyA is a substance that interferes with the endothelin metabolism [8, 10] and increases ET-1 production by both rat and human endothelial cells [9, 10] in vitro. It is also known to increase urinary [16] and plasmatic [11, 12] ET-1 levels in rats in vivo. In addition, elevated ET-1 levels were reported in non-transplant patients receiving CyA for idiopathic uveitis [13]. A potential influence of CyA on plasma ET-1 concentrations is further supported by the results of a study by Grieff et al. [17], which has shown that the dynamics of elevation of plasma ET-1 levels in humans after a single CyA dose follow those of cyclosporine.

Several previous studies have failed to demonstrate an association between plasma CyA and ET-1 levels [3, 6, 18], for which there might have been several reasons. The groups of patients in these studies were small and not well defined. The inhomogeneity of the studied patients, especially the highly differing time since transplantation, appears to be the most important point [3, 18], as Lerman's study [3] showed an increase of plasmatic ET-1 levels with time since transplantation. Grieff et al. [17] also found a higher response of ET-1 levels to CyA administration depending on the time since transplantation. Thus, these studies might have compared ET-1 concentrations of incomparable groups of patients. On the other hand, Lerman et al. [6] explored plasma ET-1 levels very early after the surgical procedure, when ET-1 concentrations can be considerably influenced by peri- or postoperative processes. Our group of patients differs from the above-mentioned in that it consists only of patients after OHTx that are an approximately equal period of time from the operation at the time of blood sample collection (368 ± 14 days).

Secondly, previous studies evaluated plasmatic concentrations of ET-1. In contrast to others, we have evaluated the levels of big ET-1 that may reflect ET-1 production more accurately then circulating ET-1. ET-1 is a peptide with high biological activity, which is very quickly cleared from circulation. According to the data in the literature, it is questionable whether ET-1 concentrations thus obtained reflect the real ET-1 production in the body exactly. In contrast, big ET-1—as a precursor of ET-1—has low biological activity, and it is cleared from circulation more slowly than ET-1. It therefore circulates in higher concentrations in the case of ET-1 overproduction. Despite that the current literature does not provide data on the influence of a single dose of CyA on big ET-1 levels (similarly to the study by Grieff et al.), we assume big ET-1 to be more suitable for estimating the exposure of the endothelin system to CyA than ET-1.

A correlation between CyA and big ET-1 plasma levels was found only in the subgroup of OHTx recipients treated with CyA-Aza-Pred, but not in the whole group of OHTx patients. This could be explained by the absence of correlation between CyA and big ET-1 levels in the MMF subgroup. The reason for this finding remains open to speculation. No differences in presence of obesity, hyperlipidemia, diabetes, hypertension, or altered renal function—i.e., factors that could influence the endothelin metabolism— were observed between the subgroups with different immunosuppressive regimens (Table 2). Graft dysfunction or acute rejection could not influence big ET-1 levels, as these represented exclusion criteria for patient enrollment to the study.

Little is known about the possible influence of other immunosuppressive agents on the ET-1 metabolism. Azathioprine was unlikely to influence big ET-1 levels in our study since all patients were treated with the same dose (1 mg/kg per day). Also, doses of prednisone did not differ very much between patients (0.09 ± 0.01 mg/kg

Table 3 Influence of some atherosclerotic risk factors on plasmatic big endothelin-1 (*ET-1*) concentrations in patients 1 year after orthotopic heart transplantation

Risk factors	Number of patients	Big ET-1 (fmol/ml)	*P*-value
Creatininemia			
< 100 μmol/l	9	14.31 ± 13.61	
> 100 μmol/l	25	13.38 ± 10.66	0.83
Cholesterolemia			
< 5.2 mmol/l	16	15.41 ± 11.45	
> 5.2 mmol/l	18	12.04 ± 11.26	0.39
Body mass index			
< 30	27	13.65 ± 11.78	
> 30	7	13.56 ± 10.05	0.98
Diabetes			
+	9	10.65 ± 8.77	
−	25	14.69 ± 12.06	0.36

per day). With regard to MMF, no report is available concerning its potential effect on the ET-1 metabolism. The fact that no correlation between CyA and big ET-1 plasma levels could be demonstrated in the group of patients treated with CyA-MMF-prednisone may be due to the unknown effect of MMF on the endothelin metabolism. Unfortunately, we were unable to study the influence of plasma levels of MMF on the levels of big ET-1. Other medication was also unlikely to influence big ET-1 levels in our study because the distribution of ACE inhibitors, beta-blockers, and statins was similar in both groups.

Since endothelin is regarded to be a marker of endothelial dysfunction, big ET-1 concentrations could be influenced by classical atherosclerotic risk factors. In contrast, we could not find any correlation between the presence of diabetes, hyperlipidemia, obesity, or altered renal function and elevated plasma big ET-1 concentrations (Table 3).

Limitations

Authors are aware of some possible limitations of the study. One of the major limitations appears to be the limited number of patients; however, our study population is numerically comparable to or even larger than those in previously published studies exploring ET-1 levels.

Conclusion

We found markedly elevated plasma big ET-1 levels in patients 1 year after successful OHTx. These elevated levels of big ET-1 correlated with plasmatic levels of CyA in the group of patients treated with an immunosuppressive regimen of CyA-Aza-Pred. The fact that an association between CyA and big ET-1 concentrations was not demonstrated in the group of patients treated with an immunosuppressive regimen consisting of CyA-MMF-Pred might be due to the possible effect of MMF on the metabolism of ET-1.

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