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ACE2 and ACE in acute and chronic rejection after human heart transplantation

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ABSTRACT

Objectives: The authors sought to evaluate cardiac activity of angiotensin-converting enzyme (ACE) and ACE2 after heart transplantation (HT) and its relation with acute rejection (AR) and chronic allograft vasculopathy (CAV).
Background: The renin-angiotensin system is altered in heart failure and HT. However, ACE and ACE2 activities in post-HT acute and chronic rejection have not been previously studied.
Methods: HT patients ($n = 45$) were included when appropriate serial endomyocardial biopsies (EMB) and coronary angiography were available for analysis. In 21 patients, three post-HT time points were selected for CAV study in EMB tissue: basal (0–3 wks), second (2–3 months) and third (4–5 months). At 10 years post-HT, CAV was evaluated by coronary angiography (CA) and patients were grouped by degree of CAV: 0–1, non-CAV ($n = 15$) and 2–3, CAV ($n = 6$). For the AR study, 28 HT patients with evidence of one EMB rejection at grade 3 and two EMB grade 1A and/or 1B rejections were selected.
Results: Post-HT, ACE2 activity was increased in the CAV group, compared to non-CAV. Patients with AR showed increased ACE, but not ACE2, activity.
Conclusions: Our results suggest that early post-HT cardiac ACE2 activity may have an important role in CAV development. In contrast, ACE activity was increased in AR. The renin-angiotensin system seems to be altered after HT and strategies to balance the system may be useful.

1. Introduction

Angiotensin-converting enzyme (ACE)-2 is a homologue of ACE. Its amino-terminal domain has 42% homology with ACE. A membrane-associated carboxypeptidase, ACE2 has one active enzymatic site and cleaves a single residue from Angiotensin (Ang)-I to generate Ang 1–9 and degrades Ang-II to the vasodilator Ang 1–7 [1,2]. ACE2 is highly expressed in the human heart, kidney, testis, and gastrointestinal system, and several studies have shown its key role in cardiorenal regulation and blood pressure control [1,2]. In addition, ACE2 protein levels are increased in failing human hearts, suggesting an important role in the negative modulation of the renin-angiotensin system (RAS), leading to the generation and degradation of angiotensin peptides after cardiac injury [3]. Circulating ACE2 activity is increased in patients with heart failure (HF) and is correlated with HF severity in both ischemic and non-ischemic cardiomyopathy. These results reinforce a putative cardioprotective and compensatory role in humans [4]. Furthermore, gene ACE2 expression in left ventricular biopsies from HF patients did not differ from healthy controls, suggesting a post-transcriptional regulation [5].

The role of ACE2 in acute myocardial infarction has been addressed in experimental studies [4,6]. There is evidence that ACE2 protein is increased in the infarct-related and peri-infarct myocardium [4]. Our group recently demonstrated that circulating ACE2 activity is increased in the acute phase of ST elevation myocardial infarction (STEMI) in

Abbreviations: ACE, angiotensin-converting enzyme; HT, heart transplantation; CAV, chronic allograft vasculopathy; AR, acute rejection; Ang, angiotensin; RAS, renin-angiotensin system; HF, heart failure; EMB, endomyocardial biopsies; LVH, left ventricle hypertrophy; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blockers.

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[3] These authors share credit for senior authorship.

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humans and correlates with the extent of jeopardized myocardium and infarct size. ACE2 activity measured acutely seems to be an independent predictor of late left ventricular(LV) systolic function, and is also associated with the occurrence of adverse remodeling [7].

Chronic rejection, also known as cardiac allograft vasculopathy (CAV), is the leading cause of late allograft loss after heart transplantation (HT). This form of vascular disease, unique to the coronary arteries of heart-transplant recipients, is observed in 30–45% of recipients by 5 years post-HT and in 50–65% by 10-year follow-up. Despite years of diagnostic and treatment efforts, CAV prevalence has not decreased, and it remains an important cause of death after the first year post-HT as well as a major indication for retransplantation [8,9]. The role of ACE2 and ACE in CAV and cardiac acute rejection (AR) has not been previously studied. Due to the RAS involvement in various cardiovascular pathologies, we hypothesized that cardiac ACE2 activity would be increased in CAV or cardiovascular mortality in post-HT human endomyocardial biopsies (EMB). In addition, we explored the potential relationship between AR episodes and cardiac ACE2 or ACE activity in post-HT human EMB.

2. Material and methods

2.1. Patient characteristics

This was a pilot study with consecutive HF patients undergoing HT ($n = 45$) at the Hospital Clínic of Barcelona from February 2000 to December 2002 were included for the CAV study if post-HT follow-up was at least 5 years and coronary arteriography, and appropriate serial EMB samples had been collected during the first year. Patients were selected for the AR study if three EMB were available, one with rejection grade 3 and two with grade 1A and/or 1B. The study was designed to compare ACE2 and ACE activities in EMB from the same patient that showed different degrees of rejection. This approach allowed us to work with paired data, adjusting for intra-patient variability while avoiding greater inter-patient variability. Thus, the control group was EMB from the same patient showing 1A and/or 1B degree of rejection. The Hospital Clinic of Barcelona Research Committee and the Ethics Committee for Clinical Research approved this study (Reference number of approval 322). Written informed consent was obtained from all research subjects.

2.2. Determination of cardiac allograft vasculopathy (CAV)

Coronary arteriography was performed in all HT patients during the first or second month post-HT, and every 2–3 years thereafter, as indicated by our clinical follow-up protocol. Patients with evidence of CAV underwent additional coronary angiography as clinically required. At 10 years post-HT, CAV was classified using the International Society of Heart and Lung Transplantation consensus criteria [10], as detailed in Supplementary information, establishing two study groups: 0–1, non-CAV patients ($n = 15$), and 2–3, CAV patients ($n = 6$). When no coronary arteriography at 10 years post-HT was available, 5-year follow-up images were reviewed. If CAV was 2 or 3 at 5 years, the patient was included in the CAV group; otherwise, the patient was excluded from the study because no conclusion could be drawn about the CAV degree at 10 years post-HT.

2.3. Determination of left ventricle hypertrophy (LVH) and heart function

The presence of LVH was defined as an end-diastolic wall thickness ≥ 12 mm, measured in the long parasternal view on a transthoracic echocardiogram. In addition, cardiac function of the transplanted heart was assessed by two-dimensional echocardiography at 10-year follow-up.

2.4. Endomyocardial biopsies

Serial EMB from the right ventricular septum were collected for all included patients after HT, following the clinical protocol for detection of AR. The EMB evaluated for the AR study were selected independently of clinical evolution. Briefly, all patients underwent EMB tissue collection and analysis for the degree of AR at the following post-HT time-points: 2 weeks, monthly up to 6 months, then at 9 months and 1 year. At least 3 serial EMB were selected for analysis of ACE and ACE2. EMB from 21 patients were used for the analysis of ACE and ACE2 activity and cardiac allograft vasculopathy (CAV). These biopsies were grouped according to the collection period, as follows: basal (first 3 weeks post-HT, mean 17 ± 2 days), second biopsy (2–3 months post-HT, mean 69 ± 5 days), and third biopsy (3–4 months post-HT, mean 126 ± 6 days).

The EMB available from 28 patients were used for the analysis of ACE and ACE2 activity and AR. A pathologist graded the EMB for degree of rejection according to the International Society of Heart and Lung Transplantation consensus criteria [11].

2.5. Immunohistochemical and immunofluorescence staining for ACE2 and ACE in heart

Four-micron-thick paraffin sections were cut from ventricular tissue. Sections were dewaxed with xylene and hydrated with decreasing alcohol concentrations. Antigens were retrieved by heating at 80 °C in 10 mM citrate at pH 6 for 40 min. (Antibodies and incubation information is provided in Supplementary information.)

2.6. ACE2 activity in endomyocardial tissue

An investigator blinded to the degree of rejection assessed ACE2 activity from the EMB. From 2 to 5 mg of endomyocardial tissue from heart-transplant recipients were homogenized and analyzed as previously described [12,13] and summarized in Supplementary information.

2.7. ACE activity in endomyocardial tissue

When enough tissue sample was available, a blinded investigator also measured ACE activity. Endomyocardial tissue from heart-transplant recipients was homogenized and analyzed as previously described [14] and summarized in Supplementary information.

2.8. Statistical analysis

Normality plots and Shapiro-Wilcoxon test were used to check normality of ACE2 and ACE activity data. The square root transformation was applied to ACE2 values after checking that they fulfilled this condition. No transformation was needed for the ACE values, although some outliers were present. Pearson coefficients were used to assess correlations between quantitative data. Fisher exact test was used for category variables.

For the CAV study, differences between CAV and non-CAV groups in enzyme activity were analyzed with repeated measures ANOVA using a general linear model procedure, including a group-by-time interaction. After this, unpaired Student *t*-test was used to compare enzyme activity at each time-point. The same analysis was applied to compare groups defined by cardiovascular mortality or left ventricular hypertrophy. Kaplan-Meier was used to estimate survival curves and survival difference was evaluated by the log-rank test.

For the AR study, the mean ACE2 or ACE activity was calculated when the patient had two EMB with the same degree of rejection (either 1A or 1B). Paired Wilcoxon signed ranks test and the Friedman test were applied.

SPSS version 20 for Windows was used. Two-sided p-values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Characteristics of the study cohort

Consecutive HT patients ($n = 45$) were considered eligible. Five patients did not have a long-term follow-up and were excluded. Of the remaining 40 patients, 12 did not have an EMB showing grade 3 rejection and could not be included in the AR study. Nineteen patients could not be included in the CAV study for the following reasons: 12 patients did not have an appropriate coronary angiography at 10 years follow-up post-HT due to lack of vascular access in 2 patients, presence of chronic kidney failure in 5 patients and the other 5 patients died between 2 months and 6 years after HT. The other 7 patients had coronary angiography at 10 years of follow-up, however there was a lack of appropriate serial EMB.

Finally, 31 patients (mean age, 52 ± 2 years; 74% men) were included in one or both studies (9 of the 40 patients were excluded from both studies and 18 were included in both studies). A detailed chart of the participant inclusion process is provided in Fig. 1S. Heart donors (74% men) had a mean age of 31 ± 2 years. Ischemic HF was diagnosed in 11 patients and non-ischemic etiology was idiopathic in 13, valvular in 4, myocarditis in 1 and amyloidosis in 2 patients. The number of patients taking an immunosuppressive treatment and the mean circulating levels of cyclosporine, tacrolimus, and mycophenolate mofetil, and the mean dose of corticosteroids, are shown in Table 1S. Four patients began cyclosporine treatment after HT and switched to tacrolimus after the first biopsy.

3.2. ACE2 and ACE localization within heart tissue

ACE2 was mainly present in cardiac muscle tissue (striated and smooth, Fig. 1A, upper panel), and both ACE2 and ACE were present in endothelial (Fig. 1C and E, respectively) and inflammatory cells.

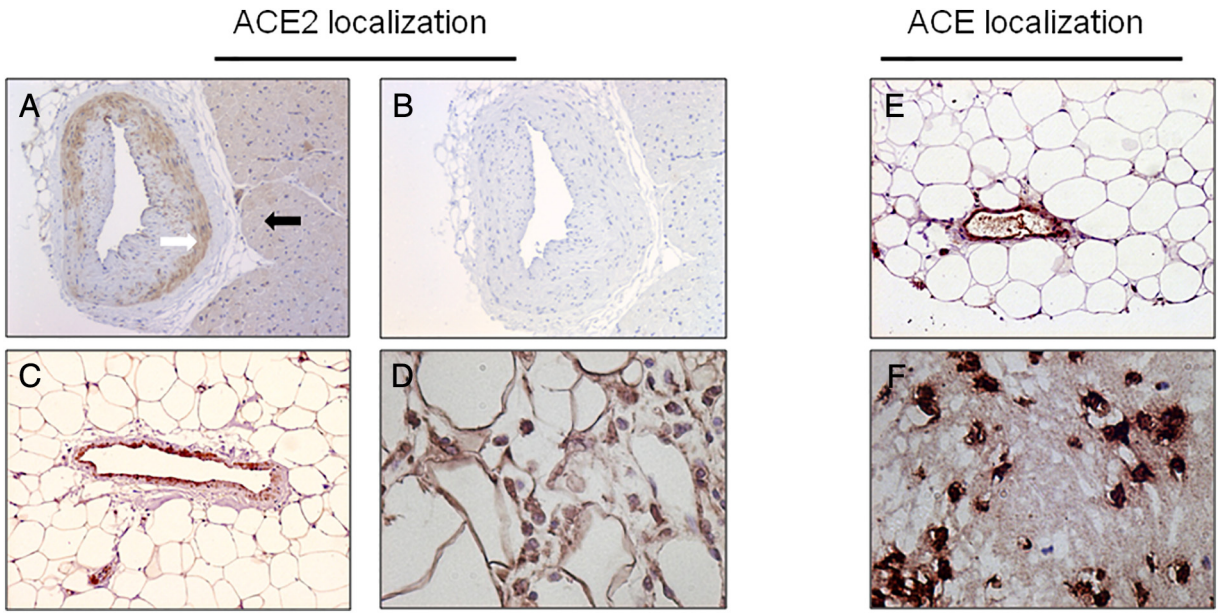

Fig. 1. Upper panel: ACE2 and ACE localization in cardiac tissue by immunohistochemistry. Localization of ACE2 in myocytes and smooth muscle cells, black and white arrow, respectively (A). Lack of staining with the preincubation of the ACE2 peptide with the ACE2 antibody (B). Localization of ACE2 in endothelial (C) and inflammatory cells (D). ACE localization in endothelial (E) and inflammatory cells (F).

The stained inflammatory cells differed in morphology, whereas ACE2-positive inflammatory cells had a lymphocyte-like morphology (Fig. 1D) and ACE-positive inflammatory cells had a mast cell-like morphology (Fig. 1F). Immunohistochemistry analysis with trypsin confirmed that the ACE-stained inflammatory cells were not mast cells (data not shown). When we used α-smooth muscle actin (α-SMA) antibody, ACE2 was localized in the arterial tunica media and in some cases colocalized with α-SMA (Fig. 2S D–E, lower panel).

Table 1
Clinical characteristics of donors and recipients associated with cardiac allograft vasculopathy (CAV) or non-CAV in heart-transplant recipients.

Demographic and clinical characteristics	Non-CAV patients ($N = 15$)	CAV patients ($N = 6$)	p
Donors			
Donor age (years)	30 ± 3	38 ± 3	ns
Cause of death (cerebrovascular/trauma), *n*	14/1	6/0	ns
Cardiac arrest (yes/no), *n*	1/14	0/6	ns
Catecholamine usage (yes/no), *n*	15/0	6/0	ns
Ischemia time (min)	183.5 ± 14	124.7 ± 14*	<0.05
Mismatch R/D sex (yes/no), *n*	6/9	3/3	ns
Blood isogroup (yes/no), *n*	15/0	6/0	ns
Recipients			
Receptor age (years)	56 ± 2	41 ± 7*	<0.05
Ischemic/non-ischemic etiology, *n*	7/8	1/5	ns
Smoking (yes/no), *n*	11/4	3/3	ns
Hypertension (yes/no), *n*	3/12	1/5	ns
Dyslipidemia (yes/no), *n*	12/3	4/2	ns
Diabetes (yes/no), *n*	2/13	1/5	ns
BSA	1.802 ± 0.056	1.808 ± 0.062	ns
WG_postHT	0.067 ± 0.483	0.667 ± 0.615	ns
Time from HT to CA (years)	7.7 ± 0.3	6.5 ± 1	ns
Cardiovascular mortality (yes/no), n	2/13	4/2*	<0.05
CMV infection (yes/no), *n*	4/11	2/4	ns
ACR-low (yes/no), *n*	11/4	4/2	ns
AMR (yes/no), *n*	0/15	0/6	ns

Ischemia time refers to the ischemia of the donor organ; Mismatch R/D sex refers to mismatched sex between the heart-transplant recipient (R) and the donor (D); BSA, body surface area calculated by the Dubois formula; WG_postHT, weight gain 4 months after heart transplantation (HT); CA, coronary angiography; CMV, *cytomegalovirus* de novo; ACR, acute cellular rejection; ACR-low, 2 or less ACR episodes during the first year post-HT (yes) and patients that had 3 or more ACR episodes (no), AMR Antibody-mediated rejection.
* indicates p<0.05.

3.3. ACE2 and ACE activity and cardiac allograft vasculopathy

Clinical characteristics of heart-transplant recipients and donors are reported in Table 1, stratified by presence or absence of CAV. The only significant differences between patients with and without CAV were younger age, shorter cold ischemia time for the heart graft, and a higher proportion of cardiovascular mortality (66%) in the CAV group.

The pattern of change over time in endomyocardial ACE2 activity differed significantly ($p < 0.05$) between the CAV and non-CAV groups (Fig. 2A–B). A rise in ACE2 activity post-HT was observed in the CAV group, with the most prominent increase at the second biopsy (61.1 ± 13.7 CAV vs 26.7 ± 5.4 RFU/μg/h non-CAV at 2–3 months, $p < 0.05$). ACE2 data is presented with a back transformation of the square root values.

Correlations between ACE2 activity and any clinical variable at the time of the second EMB in the CAV group were significantly associated with heart rate ($p < 0.05$, $r = 0.56$). Furthermore, a higher mean heart rate was found in the CAV group (87.3 ± 5 bpm) compared to the non-CAV group (73.3 ± 4.2 bpm) at this time point ($p < 0.05$). On the other hand, no differences were observed between the study groups in the proportion of patients with ≤2 AR episodes or with de novo cytomegalovirus infection (Table 1), taking immunosuppressive treatment, or in the mean circulating levels or dosage of such medication (data not shown). Finally, no significant differences were found between the CAV and non-CAV groups in ACE activity after HT (Fig. 2C–D).

3.4. ACE2 and ACE activity and cardiovascular mortality in HT patients

During the follow-up period, 6 of the 21 HT patients in the CAV study (28%) died, with 5 cardiovascular deaths related to CAV and amyloidosis as cause of death in another patient. As expected, patients with CAV had higher general and CV mortality, compared to patients without CAV (Fig. 3S). No differences in post-HT ACE2 activity or in ACE endomyocardial activity were associated with cardiovascular death (data not shown).

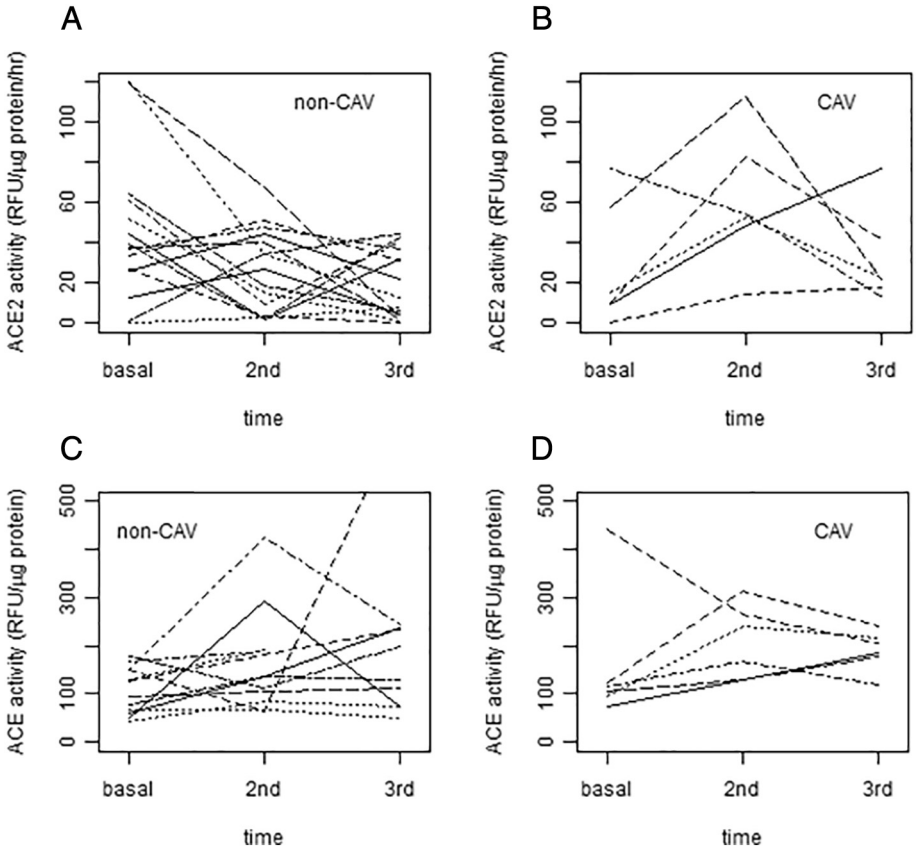

Fig. 2. ACE2 activity in serial endomyocardial biopsies (EMB) at three time-points post-heart transplantation: 1st (basal), 2nd (2–3 months), 3rd (4–5 months) in non-CAV patients (A) and in CAV patients (B). ACE activity in serial endomyocardial biopsies (EMB) at the same three time-points in non-CAV patients (C) and in CAV patients (D).

3.5. ACE2 and ACE activity and left ventricular hypertrophy in HT patients

During the follow-up period, 14 of 21 HT patients (67%) were diagnosed of LVH. There were no differences in ACE2 or ACE endomyocardial activity between LVH and non-LVH HT (data not shown).

3.6. ACE2 and ACE activity and acute rejection in HT patients

No differences were found in ACE2 activity based on the degree of rejection shown by the EMB: 1A, 43.7 ± 8.2 RFU/μg/h ($n = 18$); 1B, 31.1 ± 6.4 RFU/μg/h ($n = 22$); 3, 32.84 ± 4.5 ($n = 28$) (Fig. 3A). In addition, no differences were found in intra-patient comparison of cardiac ACE2 activity in EMBs with all three AR degrees. In analysis of EMB, ACE activity with rejection degree 3 ($n = 24$) was greater than rejection degree 1A ($n = 18$) (182 ± 17.8 vs 123.5 ± 12.2 RFU/μg, respectively, $p = 0.011$) (Fig. 3B). No differences were found between ACE activity in EMB with degree 1B ($n = 21$) (178.8 ± 25.4) and EMB with degrees 1A or 3. Finally, no intra-patient differences were observed in cardiac ACE activity of EMBs with all three AR degrees. Notably, none of the HT patients were diagnosed of acute mediated rejection.

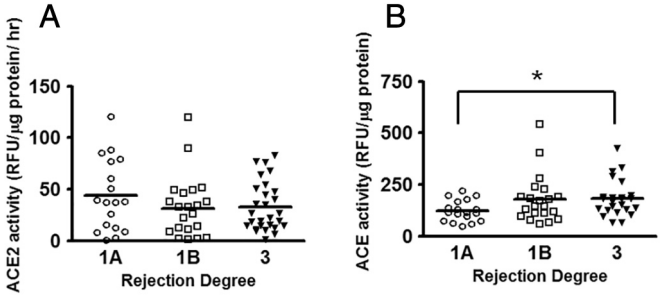

Fig. 3. ACE2 activity (A) and ACE activity (B) in endomyocardial biopsies (EMB) with no acute rejection (1A and 1B) and acute rejection with a degree of 3. *$p < 0.05$.

3.7. ACE2 and ACE activity and RAS system treatment in HT patients

The first data analysis highlighted the presence of very high levels of ACE activity in some EMB. Not surprisingly, the corresponding patients were treated with angiotensin II receptor blockers (ARBs) at the time of the EMB harvest. Thus, patients taking ARB showed significantly increased ACE activity as compared to non-ARB treated patients (3811.21 ± 1605.36 vs. 192.58 ± 25.34 RFU/μg of protein, $p < 0.01$). On the other hand, no differences in ACE activity were found between patients not treated or treated with ACEIs (199.45 ± 27.41 vs 108.89 ± 17.25 RFU/μg of protein). No differences in ACE2 activity were observed based on ARB or ACEI therapy.

4. Discussion

The present pilot study shows that alterations in cardiac levels of ACE and ACE2 may be relevant to understanding the role of the RAS in acute and chronic rejection in HT patients. Cardiac ACE2 activity is upregulated in early post-HT in patients with chronic rejection. In AR, ACE2 is not modified but cardiac ACE activity is increased. Our results reinforce the differing roles of these enzymes within the heart; ACE2 activity may be an early marker of CAV, whereas increased ACE may

indicate acute heart rejection. Thus, the study of both enzymes may help to assess HT patients at risk for acute or chronic rejection.

CAV pathophysiology involves immunologic factors, such as endothelial antibody-mediated damage, and non-immunologic factors, such as vascular risk factors, cytomegalovirus infection, or ischemia-reperfusion injury [15–18]. It is characterized by diffuse luminal narrowing, secondary to neointimal hyperplasia, concentric medial microvascular disease, and impaired coronary blood flow [19–21]. Nevertheless, CAV progression after HT is not fully understood. Among the recognized CAV triggering factors, receptor age and ischemia time were lower in the CAV group than in the non-CAV patients [22–25]. A trend to higher donor age was detected in CAV patients, but it did not reach statistical significance.

Yousufuddin et al. demonstrated that cardiac Ang-II receptors have a role in the progression of intimal thickening and the development of transplant vasculopathy in heart-transplant recipients. Both Ang-II type 1 and type 2 receptor gene transcripts in the transplanted heart predict a change in maximal intimal thickness ≥ 0.3 mm over one year in heart-transplant recipients [26]. These data suggest evidence for a potential role of cardiac Ang-II receptor gene transcripts, particularly of type 1, in the development of transplant vasculopathy in heart-transplant recipients. The present evidence suggests extending the RAS concept of conventional atherosclerosis to transplant vasculopathy and supports a role of non-immunological factors in the pathogenesis of the latter. Interestingly, we found that ACE2 activity was directly correlated with heart rate. In HT patients, elevated heart rate is due to surgical denervation and has been associated to CAV-related mortality [27]. These results suggest that increased ACE2 activity coupled with increased heart rate may indicate a patient with increased CAV risk after HT.

In the heart, ACE2 mainly cleaves Ang-II, a vasoconstrictive, proinflammatory, and fibrogenic peptide, to Ang-(1–7), a peptide with opposed biological actions [1,2,28]. ACE2 deletion in mice causes increased levels of Ang-II and systolic dysfunction that is restored by ACE deletion, suggesting that the deleterious effect is mediated by an increase in Ang-II peptide [29]. Elevated plasma ACE2 activity has been observed in HF patients, in the acute phase of myocardial infarction, and in patients with previous history of myocardial infarction [7,13,30]. ACE2 expression has been previously demonstrated in experimental models and human heart [4,31]. In human heart, ACE2 is expressed in cardiomyocytes, vascular endothelium, and smooth muscle cells, and is increased in the infarct-related and peri-infarct myocardium [4,32]. Our group previously studied ACE2 gene expression in left ventricular biopsies from patients with HF undergoing HT and were not able to find alterations in human end-stage HF [5]. The present finding that cardiac ACE2 activity was upregulated in early post-HT in patients with CAV, observed directly in samples from heart biopsies in HT patients, suggests imbalance of RAS within the human heart as a potential biomarker of risk for chronic vasculopathy. Given that ACE2 activity is easily measurable in serum samples and is increased in various pathological conditions, one may extrapolate that the study of circulating ACE2 activity in HT will mirror the results obtained in heart biopsies.

Acute cellular rejection (ACR or AR) is a common problem after HT, particularly in the first 6 months, and is predominantly T-cell mediated. Approximately 15% to 25% of HT patients will experience at least one ACR episode in the first postoperative year [9]. Meanwhile, in low AR grades such as 1A and 1B, perivascular and interstitial mononuclear cells are present whereas in the higher (grade 3) acute cellular rejection, macrophages and polymorphous cells are also detected in the heart biopsy associated with areas of myocyte damage [33]. Our immunohistochemical analysis of ACE reactivity in biopsy samples showed a strong staining of polynuclear inflammatory cells. ACE expression in inflammatory cells such as macrophages has been previously reported [34]; however, high ACE expression in non-macrophage inflammatory cells also has been reported and is in agreement with our ACE localization results [35]. The consequences of having high ACE should be further explored and could be related to the increased fibrosis detected in hearts from HT patients [36,37].

5. Limitations

The main limitations of the study are the small sample size and the limited amount of sample tissue available, thus the study can be considered a pilot study with preliminary data. Thus, we began by measuring ACE2 activity, the relatively new enzyme, and followed by measuring the well-known ACE enzyme. With this strategy, ACE activity was measured in a smaller number of patients than was ACE2 heart activity.

Another limitation is the high number of transplanted patients that did not fulfill all the requirements for the follow-up studies. In the AR study, some patients did not have a grade 3 EMB, and in the CAV study some patients did not have an appropriate coronary arteriography. Therefore, from the 45 potential patients transplanted during the inclusion period, only 31 individuals were included, a total of 28 in the AR study and 21 in the CAV study.

6. Conclusions

Our study performed in serial EMB after HT demonstrates that both ACE2 and ACE enzymes are altered in heart transplant recipients, in chronic or acute heart allograft rejection, respectively. This finding suggests that both enzymes are potential early biomarkers of cardiac allograft dysfunction. Heart RAS is complex and it is altered after HT. Thus, strategies aimed to balance the cardiac RAS system may be useful to increase the lifespan of allograft transplants.

Clinical perspectives: competencies in medical knowledge and translational outlook

The present study shows that alterations in cardiac levels of ACE and ACE2 may be relevant to understanding the role of the RAS in acute and chronic rejection of a heart transplant. In early post-HT, cardiac ACE2 is up-regulated in patients with chronic rejection, whereas ACE is increased in AR. Our results suggest that ACE2 and ACE are relevant to CAV and AR. Thus, early strategies to balance the RAS may be useful in HT patients.

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Disclosure

The authors of this manuscript have no conflicts of interest.

Appendix A. Supplementary data

Materials and methods are detailed in the supplementary information document. Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2018.10.002.

References

[1] M.A. Crackower, R. Sarao, G.Y. Oudit, C. Yagil, I. Kozieradzki, S.E. Scanga, et al., Angiotensin-converting enzyme 2 is an essential regulator of heart function, Nature 417 (2002) 822–828.

[2] M. Donoghue, F. Hsieh, E. Baronas, K. Godbout, M. Gosselin, N. Stagliano, et al., A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9, Circ. Res. 87 (5) (2000) e1–e9.

[3] L.M. Burrell, C.I. Johnston, C. Tikellis, M.E. Cooper, ACE2, a new regulator of the renin–angiotensin system, Trends Endocrinol. Metab. 15 (4) (2004 May) 166–169.

[4] L.M. Burrell, J. Risvanis, E. Kubota, R.G. Dean, MacDonald PS, S. Lu, et al., Myocardial infarction increases ACE2 expression in rat and humans, Eur. Heart J. 26 (4) (2005 Feb) 369–375 (discussion 322–4).

[5] M. Batlle, E. Roig, F. Perez-Villa, S. Lario, P. Cejudo-Martin, E. García-Pras, et al., Increased expression of the renin-angiotensin system and mast cell density but not of angiotensin-converting enzyme II in late stages of human heart failure, J. Heart Lung transplant. Off. Publ. Int. Soc. Heart Transplant. 25 (9) (2006 Sep) 1117–1125.

[6] C.M. Ferrario, Myocardial infarction increases ACE2 expression in rat and humans, Eur. Heart J. 26 (11) (2005 Jun) 1141 (author reply 1141-3).

[7] J.T. Ortiz-Pérez, M. Riera, X. Bosch, T.M. De Caralt, R.J. Perea, J. Pascual, et al., Role of circulating angiotensin converting enzyme 2 in left ventricular remodeling following myocardial infarction: a prospective controlled study, PLoS One 8 (4) (2013 Jan), e61695.

[8] S. Agarwal, A. Parashar, S.R. Kapadia, E.M. Tuzcu, D. Modi, R.C. Starling, et al., Long-term mortality after cardiac allograft vasculopathy: implications of percutaneous intervention, JACC Heart Fail. 2 (3) (2014 Jun) 281–288.

[9] L.H. Lund, L.B. Edwards, A.I. Dipchand, S. Goldfarb, A.Y. Kucheryavaya, B.J. Levvey, et al., The Registry of the International Society for Heart and Lung Transplantation: thirty-third adult heart transplantation report—2016; focus theme: primary diagnostic indications for transplant, J. Heart Lung Transplant. 35 (10) (2016 Oct) 1158–1169.

[10] M.R. Mehra, M.G. Crespo-Leiro, A. Dipchand, S.M. Ensminger, N.E. Hiemann, J.A. Kobashigawa, et al., International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010, J. Heart Lung Transplant. Off. Publ. Int. Soc. Heart Transplant. 29 (7) (2010 Jul) 717–727.

[11] M.E. Billingham, N.R. Cary, M.E. Hammond, J. Kemnitz, C. Marboe, H.A. McCallister, A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: heart rejection study group, Int. Soc. Heart Transplant. J. Heart Transplant. 9 (6) (1990) 587–593.

[12] L. Anguiano, M. Riera, J. Pascual, J.M. Valdivielso, C. Barrios, A. Betriu, et al., Circulating angiotensin-converting enzyme 2 activity in patients with chronic kidney disease without previous history of cardiovascular disease, Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transplant Assoc. Eur. Renal Assoc. 30 (7) (2015 Jul) 1176–1185.

[13] M.J. Soler, M. Riera, M. Crespo, M. Mir, E. Márquez, M.J. Pascual, et al., Circulating angiotensin-converting enzyme 2 activity in kidney transplantation: a longitudinal pilot study, Nephron Clin. Pract. 121 (3–4) (2012 Jan) c144–c150.

[14] S.L. Schwager, A.K. Carmona, E.D. Sturrock, A high-throughput fluorimetric assay for angiotensin I-converting enzyme, Nat. Protoc. 1 (4) (2006 Nov) 1961–1964.

[15] A. Loupy, C. Toquet, P. Rouvier, T. Beuscart, M.C. Bories, S. Varnous, et al., Late failing heart allografts: pathology of cardiac allograft vasculopathy and association with antibody-mediated rejection, Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transplant Surg. 16 (1) (2016 Jan) 111–120.

[16] J.A. Kobashigawa, R.C. Starling, M.R. Mehra, R.L. Kormos, G. Bhat, M.L. Barr, et al., Multicenter retrospective analysis of cardiovascular risk factors affecting long-term outcome of de novo cardiac transplant recipients, J. Heart Lung Transplant. Off. Publ. Int. Soc. Heart Transplant. 25 (9) (2006 Sep) 1063–1069.

[17] J.F. Delgado, A.G. Reyne, S. de Dios, F. López-Medrano, A. Jurado, R.S. Juan, et al., Influence of cytomegalovirus infection in the development of cardiac allograft vasculopathy after heart transplantation, J. Heart Lung Transplant. 34 (8) (2015 Aug) 1112–1119.

[18] J.Y. Lim, S.H. Jung, M.S. Kim, J.-J. Park, T.-J. Yun, J.J. Kim, et al., Cardiac allograft vasculopathy after heart transplantation: is it really ominous? Clin. Transpl. 31 (2) (2017 Feb), e12883.

[19] E.M. Tuzcu, A.C. De Franco, M. Goormastic, R.E. Hobbs, G. Rincon, C. Bott-Silverman, et al., Dichotomous pattern of coronary atherosclerosis 1 to 9 years after transplantation: insights from systematic intravascular ultrasound imaging, J. Am. Coll. Cardiol. 27 (4) (1996 Mar) 839–846.

[20] N.E. Hiemann, E. Wellnhofer, C. Knosalla, H.B. Lehmkuhl, J. Stein, R. Hetzer, et al., Prognostic impact of microvasculopathy on survival after heart transplantation: evidence from 9713 endomyocardial biopsies, Circulation 116 (11) (2007 Sep) 1274–1282.

[21] K.F. Kofoed, J. Czernin, J. Johnson, J. Kobashigawa, M.E. Phelps, H. Laks, et al., Effects of cardiac allograft vasculopathy on myocardial blood flow, vasodilatory capacity, and coronary vasomotion, Circulation 95 (3) (1997 Feb) 600–606.

[22] S. Arora, A. Gunther, B. Wennerblom, T. Ueland, A.K. Andreassen, E. Gude, et al., Systemic markers of inflammation are associated with cardiac allograft vasculopathy and an increased intimal inflammatory component, Am. J. Transplant. 10 (6) (2010 May 10) 1428–1436.

[23] J.M. Aranda, J. Hill, Cardiac transplant vasculopathy, Chest 118 (6) (2000 Dec) 1792–1800.

[24] E. Roig, L. Almenar, M. Crespo-Leiro, J. Segovia, S. Mirabet, J. Delgado, et al., Heart transplantation using allografts from older donors: multicenter study results, J. Heart Lung Transplant. 34 (6) (2015 Jun) 790–796.

[25] D. Schmauss, M. Weis, Cardiac allograft vasculopathy: recent developments, Circulation 117 (16) (2008 Mar 31) 2131–2141.

[26] M. Yousufuddin, S. Haji, R.C. Starling, E.M. Tuzcu, N.B. Ratliff, D.J. Cook, et al., Cardiac angiotensin II receptors as predictors of transplant coronary artery disease following heart transplantation, Eur. Heart J. 25 (5) (2004 Mar) 377–385.

[27] M.Á. Castel, E. Roig, J. Rios, C. Tomas, S. Mirabet, M. Cardona, et al., Long-term prognostic value of elevated heart rate one year after heart transplantation, Int. J. Cardiol. 168 (3) (2013 Oct 3) 2003–2007.

[28] V.B. Patel, J.-C. Zhong, M.B. Grant, G.Y. Oudit, Role of the ACE2/angiotensin 1-7 axis of the renin-angiotensin system in heart failure, Circ. Res. 118 (8) (2016 Apr) 1313–1326.

[29] P.J. Garabelli, J.G. Modrall, J.M. Penninger, C.M. Ferrario, M.C. Chappell, Distinct roles for angiotensin-converting enzyme 2 and carboxypeptidase A in the processing of angiotensins within the murine heart, Exp. Physiol. 93 (5) (2008 May) 613–621.

[30] S. Epelman, K. Shrestha, R.W. Troughton, G.S. Francis, S. Sen, A.L. Klein, et al., Soluble angiotensin-converting enzyme 2 in human heart failure: relation with myocardial function and clinical outcomes, J. Card. Fail. 15 (7) (2009 Sep) 565–571.

[31] H. Roca-Ho, M. Riera, V. Palau, J. Pascual, M. Soler, Characterization of ACE and ACE2 expression within different organs of the NOD mouse, Int. J. Mol. Sci. 18 (3) (2017 Mar) 563.

[32] I. Hamming, M.E. Cooper, B.L. Haagmans, N.M. Hooper, R. Korstanje, A.D. Osterhaus, et al., The emerging role of ACE2 in physiology and disease, J. Pathol. 212 (1) (2007) 1–11.

[33] S. Stewart, G.L. Winters, M.C. Fishbein, H.D. Tazelaar, J. Kobashigawa, J. Abrams, et al., Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection, J. Heart Lung Transplant. 24 (11) (2005 Nov) 1710–1720.

[34] R.M. Wösten-van Asperen, R. Lutter, P.A.C. Specht, J.B. van Woensel, C.M. van der Loos, S. Florquin, et al., Ventilator-induced inflammatory response in lipopolysaccharide-exposed rat lung is mediated by angiotensin-converting enzyme, Am. J. Pathol. 176 (5) (2010 May) 2219–2227.

[35] F. Ribichini, F. Pugno, V. Ferrero, G. Bussolati, M. Feola, P. Russo, et al., Cellular immunostaining of angiotensin-converting enzyme in human coronary atherosclerotic plaques, J. Am. Coll. Cardiol. 47 (6) (2006 Mar) 1143–1149.

[36] P. Pedrotti, E. Bonacina, C. Vittori, M. Frigerio, A. Roghi, Pathologic correlates of late gadolinium enhancement cardiovascular magnetic resonance in a heart transplant patient, Cardiovasc. Pathol. 24 (4) (2015 Jul) 247–249.

[37] M. Pichler, P.P. Rainer, S. Schauer, G. Hoefler, Cardiac fibrosis in human transplanted hearts is mainly driven by cells of intracardiac origin, J. Am. Coll. Cardiol. 59 (11) (2012 Mar 13) 1008–1016.