

## 90-kDa N-domain angiotensin I-converting enzyme (ACE): possible marker for hypertension in a renal transplant model

Enzima conversora de angiotensina I (ECA) N-domínio 90-kDa: possível marcador para hipertensão em modelo de transplante renal

### Authors

Cleber A Leite<sup>1</sup>  
 Nádia SC Bertocello<sup>1</sup>  
 Ingrid KM Watanabe<sup>1</sup>  
 Fernanda B Fernandes<sup>1</sup>  
 Maria Claudina C Andrade<sup>1</sup>  
 Fernanda A Ronchi<sup>1</sup>  
 Danielle Y Arita<sup>1</sup>  
 Fernanda K Marcondes<sup>2</sup>  
 Tatiana S Cunha<sup>1</sup>  
 Dulce E Casarini<sup>1</sup>

<sup>1</sup> Universidade Federal de São Paulo.

<sup>2</sup> Universidade Estadual de Campinas.

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### Correspondence to:

Dulce E Casarini.  
 Divisão de Nefrologia,  
 Departamento de Medicina.  
 Rua Botucatu, nº 740, Vila  
 Clementino, São Paulo, SP,  
 Brazil.  
 E-mail: casarini.elena@  
 unifesp.br

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### ABSTRACT

**Introduction:** Hypertension is nearly universal in kidney transplant and several factors are associated with post transplant hypertension, including immunosuppressive medications and genetic predisposition. **Objective:** The aims were to evaluate the effects of spontaneously hypertensive rats (SHR) kidney transplantation in Wistar rats and the possible transference of 80/90-kDa N-domain ACE. **Methods:** To do so, the data from Wistar recipients of kidney from SHR were compared to data from transplanted Wistar submitted to CsA treatment and, to Wistar Sham. **Results and Discussion:** Despite the unaltered blood pressure observed at early stages, 80/90-kDa ACE was found expressed in the urine of rats 7 and 15 days after transplantation, which was intense when rats became hypertensive 30 days post-surgery. **Conclusion:** Our data show that this enzyme is associated with the development of hypertension, and this marker appears in the urine before any substantial blood pressure alteration.

**Keywords:** hypertension; kidney transplantation; renin-angiotensin system.

### RESUMO

**Introdução:** A hipertensão é altamente prevalente pós-transplante renal e vários fatores estão associados incluindo o tratamento com imunossuppressores e a predisposição genética. **Objetivo:** Os objetivos foram avaliar os efeitos do transplante do rim de ratos espontaneamente hipertensos (SHR) em ratos Wistar, e a possível transferência da ECA N-domínio de 80/90-kDa para os tecidos dos receptores. **Métodos:** Para isso, os dados dos animais Wistar receptores dos rins de SHR foram comparados aos dados dos Wistar submetidos ao tratamento com CsA e Wistar Sham. **Resultados e Discussão:** Apesar da pressão arterial permanecer inalterada nos estágios iniciais pós-transplante renal, a expressão da ECA de 80/90-kDa foi identificada na urina de ratos 7 e 15 dias após o transplante, e de forma mais intensa aos 30 dias após a cirurgia, quando os animais tornaram-se hipertensos. **Conclusão:** Nossos dados mostram que ECA N-domínio está associada ao desenvolvimento da hipertensão, e que este marcador pode ser identificado na urina pós-transplante renal antes mesmo de qualquer alteração da pressão arterial.

**Palavras-chave:** hipertensão; sistema renina-angiotensina; transplante de rim.

### INTRODUCTION

Hypertension is nearly universal in kidney transplant recipients and its results from the interaction of several factors, including immunosuppressive medications, graft dysfunction, as well as genetic predisposition.<sup>1</sup>

Although it has been demonstrated that the increase in blood pressure post transplant happens in 90% of cases secondary to immunosuppressive treatment, as for instance, cyclosporine (CsA),<sup>2</sup> it remains one of the pivotal drug to prevent renal allograft rejection.

The exact mechanisms of CsA-induced hypertension remain obscure, but several lines of evidence suggest an involvement of the renin-angiotensin system (RAS).<sup>3</sup>

The characterization of 80/90-kDa N-domain angiotensin converting enzyme (ACE) as a possible biological marker of hypertension, led us to hypothesize that this protein could be associated to the development of hypertension in kidney transplant.<sup>4-6</sup> Therefore, the present study was designed to investigate: 1) the effects of SHR kidney transplantation in Wistar rats

and the possible transference of 80/90-kDa ACE isoform and 2) whether this transference might contribute to the development of CsA-induced hypertension.

## MATERIAL AND METHODS

### GENERAL PROCEDURES

Male Wistar and spontaneously hypertensive rats (SHR), 4-months-old, were randomly assigned into following groups (n = 6/group): 1) Sham-control group: Wistar rats which had kidneys removed, re-transplanted in the same compartment and were euthanized after 7, 15 or 30 days (C-7, C-15 and C-30); 2) Transplanted group: Wistar rats which received a kidney from SHR and were euthanized 7, 15 or 30 days after transplantation (T-7, T-15 and T-30); 3) Transplanted group treated with CsA: Wistar rats which received a kidney from SHR and were treated with a daily intraperitoneal dose of CsA (2mg/Kg/day) during 7, 15 and 30 days after surgery (TCsA-7, TCsA-15 and TCsA-30). The protocols were approved by the Institutional Review Board of Federal University of São Paulo, Brazil (number 04348/02).

### KIDNEY TRANSPLANTATION

The left kidneys from SHR rats were orthotopically transplanted into male Wistar recipients following ipsilateral nephrectomy under anesthesia. Briefly, donor left kidney was removed and placed in cold saline. After clamping renal vessels, native recipient left kidney was removed, replaced by the donor kidney and end-to-end anastomoses were performed for renal vessels and ureter.<sup>7</sup>

### SYSTOLIC BLOOD PRESSURE ANALYSIS

Systolic arterial blood pressure was measured in conscious rats, using a computerized tail-cuff system (BP-2000, Blood Pressure Analysis System™, Visitech System)<sup>8</sup> on the 7<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup> days after surgical manipulation or renal transplant.

### URINE AND TISSUE COLLECTION

On the 7<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup> days after surgical manipulation or renal transplant, rats were placed in metabolic cages, during 24-hour for urine collection. At the end of protocol, rats were euthanized by decapitation and trunk blood and organs were collected. Kidneys and lung were homogenized<sup>9</sup> and protein concentration was determined by Bradford method.<sup>10</sup>

### ACE DETECTION BY WESTERN BLOTTING

Electrophoresis was performed according to Laemmli method.<sup>11</sup> The bands were revealed using substrates nitroblue tetrazolium chloride/4-bromine-3chloro-inlyl phosphatate (NBT/BCIP).

### NH<sub>2</sub>-TERMINAL SEQUENCE OF PURIFIED ACE 80/90 KDA ISOFORM

the NH<sub>2</sub>-terminal sequence of ACEs was deduced from amino acid sequencing using a protein sequencer (model PPSQ-23, Shimatzu).<sup>12</sup>

### ENZYMATIC ACTIVITY ASSAY

ACE catalytic activity was determined as described by Friedland and Silverstein,<sup>13</sup> using Z-Phe-His-Leu as substrate.<sup>12</sup>

### STATISTICAL ANALYSIS

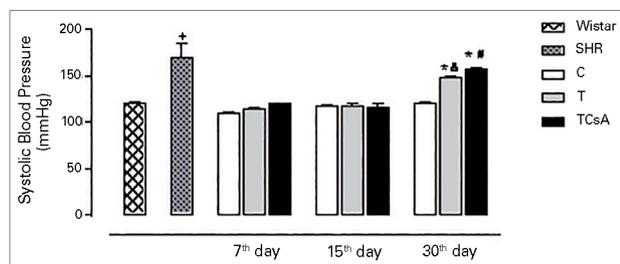
Results are presented as means ± standard error of mean (SEM). Data were evaluated by 1-way ANOVA with Fisher's significant difference test. A *p*-value of < 0.05 was considered to be significant.

## RESULTS AND DISCUSSION

Multiple causes may contribute to post-transplantation hypertension. Regarding donor factors these include not only pre-existing hypertension, and poor allograft quality, but also genetic factors. In this context, we have described the presence of 80/90-kDa ACE isoform in urine/kidney from individuals predisposed to hypertension, and hypertensive patients and animals.<sup>4-6</sup> So, we aimed to evaluate if the transference of the ACE isoform occurs when a renal graft from a SHR is transplanted to a normotensive rat, how it could affect the RAS and correlate SBP with the presence of 80/90-kDa ACE in urine.

SHR presented significantly increased SBP compared to Wistar rats (171 ± 8 *vs.* 120 ± 2 mmHg, *p* < 0.05, Figure 1). On both the 7<sup>th</sup> and 15<sup>th</sup> days after transplantation, SBP of recipients of SHR kidney was similar to C and Wistar rats, and significantly lower than SHR (Figure 1). In contrast, 30 days after SHR kidney transplantation, T group presented significantly increased SBP (*versus* C and Wistar rats), similar to SHR (Figure 1). Moreover, results show that CsA treatment did not influence SBP 7, 15 or 30 days after renal transplantation. Corroborating with our findings, Kawabe *et al.*<sup>14</sup> and Rettig *et al.*<sup>15</sup> showed that SBP in

**Figure 1.** Baseline SBP of SHR and Wistar transplanted rats after 7, 15 or 30 days of surgical manipulation or kidney transplantation, treated or not with cyclosporine (CsA, 2mg/Kg/day; n = 6/group). Values are presented as mean  $\pm$  SEM. SHR: spontaneously hypertensive rats (SHR), C: Wistar sham control, T: Wistar rats transplanted with kidney from SHR, TCsA: Wistar rats transplanted with kidney from SHR and treated with CsA. + vs. all experimental groups; \* vs. C-30; & vs. T-7 and T-15; # vs. TCsA-7 and TCsA-15;  $p < 0.05$ .



renal transplanted rats is strongly determined by the the genetic background of the transplanted kidney.

According to Figure 2, urine from Wistar rats recipients of SHR kidney present three isoforms of ACE: somatic isoform with 190-kDa, N-domain isoform with 65-kDa, and also the 80/90-kDa ACE isoform. The 80/90-KDa ACE was detected in urine from SHR and also in urine from T group, 7 days after SHR transplantation (T-7), with increased expression on the 30<sup>th</sup> day (T-30; Figure 2).

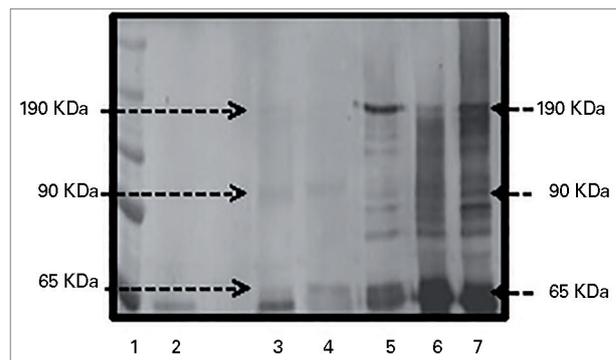
It is important to highlight that Wistar rats usually do not express this isoform as described by Ronchi *et al.*<sup>4</sup> Considering that the detection of 80/90-kDa ACE isoform in urine of T-7 and T-15 groups occurred despite the unaltered SBP, results demonstrate that the protein message is transferred and predisposes these animals to hypertension.

The transplantation seems to affect the expression of the 80/90-kDa isoform in transplanted kidney, which was higher in the group untreated with CsA as compared with the treated one (Figure 3, lane 1 vs. Lane 6), indicating that this isoform could be intensely affected by an immune response to the transplant.

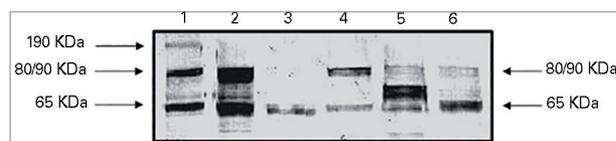
In addition to the immune cells transferred with the renal graft, native macrophages and other immune cells carrying the genetic message of 80/90-kDa ACE, transferred from the grafted kidney, could migrate to the transplanted organ, increasing locally the isoform expression. This hypothesis is supported by the observation that 80/90-kDa ACE expression decreased under CsA therapy.

The NH<sub>2</sub>-terminal sequence of purified of ACE isoform with 80/90-kDa from transplanted kidney is shown in Table 1. The sequencing showed that 80/90-kDa ACE isoform from native kidney of T-30 and

**Figure 2.** Analysis of the expression of ACE isoforms in the urine of SHR and Wistar transplanted rats after 7, 15 or 30 days (anti-ACE antibody 9B9). Lane 1: Molecular weight standard, Lane 2: C-30, Lane 3: SHR; Lane 4: T-7, Lane 5: T-15, Lane 6: T-21, Lane 7: T-30.



**Figure 3.** Analysis of the expression of ACE isoform in renal tissue of SHR, Wistar Sham C and T-30 days (anti-ACE antibody 9B9). Lane 1: Native kidney of T-30; Lane 2: SHR; Lane 3: Wistar; Lane 4: Native kidney of C-30; Lane 5: renal allograft of T-30; Lane 6: Native kidney of TCsA-30.



**TABLE 1** NH<sub>2</sub>-TERMINAL SEQUENCES OF 80/90-KDA ACE ISOFORM AND ALIGNMENT WITH NH<sub>2</sub>-TERMINAL SEQUENCES OF BOVINE, MOUSE, RAT, AND HUMAN ACE

| Groups                    | Sequence    |
|---------------------------|-------------|
| Native kidney T-30 ACE    | DPGXQPGNXS  |
| Native kidney TCsA-30 ACE | - PGXQPGNFS |
| Bovine ACE                | DPALQPGNF - |
| Mouse ACE                 | DPGTQPSNF - |
| Rat ACE                   | DPGLQPGNFS  |
| Human ACE                 | DPGLQPGNFS  |

TCsA-30 groups is similar to somatic rat, mouse, and human ACEs (~80% homology), proving that the detected enzymes contain the NH<sub>2</sub>-terminal portion of the molecule (Table 1).

ACE activity in native kidney of transplanted rats was similar among the groups. Moreover, renal ACE activity of native kidney of T-15, TCsA-15, T-30 and TCsA-30 groups were higher than SHR group. It is also important to mention that lower ACE activity was observed in transplanted kidneys on the 15<sup>th</sup> day (T-15, TCsA-15) as compared with Wistar, C and SHR, with no effect of CsA. Interestingly, on the 30<sup>th</sup> day after transplantation, ACE activity of transplanted kidney (T-30, TCsA-30) was not statistically different from SHR (Table 2).

**TABLE 2** ACE ACTIVITY IN LUNG, NATIVE KIDNEY AND TRANSPLANTED KIDNEY OF SHR AND WISTAR TRANSPLANTED RATS AFTER 7, 15 OR 30 DAYS OF SURGICAL MANIPULATION OR KIDNEY TRANSPLANTATION, TREATED OR NOT WITH CYCLOSPORINE (CsA, 2MG/KG/DAY; N = 6/GROUP).

| Groups  | Lung (nmol/mL/min)        | Native Kidney (nmol/mL/min) | Transplanted Kidney (nmol/mL/min) |
|---------|---------------------------|-----------------------------|-----------------------------------|
| Wistar  | 260 ± 19                  | 24 ± 5                      | –                                 |
| SHR     | 478 ± 29 <sup>+</sup>     | 21 ± 1                      | –                                 |
| C       | 290 ± 14                  | 27 ± 4                      | –                                 |
| T-15    | 404 ± 26 <sup>#</sup>     | 35 ± 5                      | 10 ± 1                            |
| TCsA-15 | 257 ± 20 <sup>&amp;</sup> | 31 ± 5                      | 8 ± 1                             |
| T-30    | 296 ± 10 <sup>&amp;</sup> | 31 ± 4                      | 16 ± 1 <sup>&amp;</sup>           |
| TCsA-30 | 232 ± 15 <sup>*</sup>     | 32 ± 2                      | 21 ± 3 <sup>*</sup>               |

Values are presented as mean ± SEM (n = 6/group). <sup>+</sup> vs. all experimental groups, <sup>#</sup> vs. Wistar and C, <sup>&</sup> vs. T-15; <sup>\*</sup> vs. T-30; *p* < 0.05.

The pulmonary ACE activity was increased in T-15 compared to Wistar, suggesting that renal transplantation induced this alteration, but it was not enough to increase SBP of recipient. CsA therapy significantly reduced pulmonary ACE activity on the 15<sup>th</sup> and on the 30<sup>th</sup> day after transplantation (T-15 vs. TCsA-15 and T-30 vs. TCsA-30; Table 2). Our results suggest that the transplantation of SHR kidney into Wistar has also a short-term effect on ACE activity in the primary source of ACE production (lung), which can be prevented by immunosuppressive therapy.

The early detection of 80/90-kDa isoform in urine of transplanted rats indicates that this biological marker of hypertension appears before any substantial BP increase. Our data contribute to highlight the relationship between tissue RAS and graft dysfunction.

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