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Local Angiotensin-Converting Enzyme 2 Gene Expression in Kidney Allografts Is Not Affected by Renin-Angiotensin-Aldosterone Inhibitors

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Keywords
COVID-19 · Angiotensin-converting enzyme 2 · Renin-angiotensin system inhibitors · Kidney

Abstract
Background: Preclinical studies suggested that pharmacological inhibition of the renin-angiotensin-aldosterone system (RAAS) by ACE inhibitors (ACEis) or angiotensin II receptor blockers (ARBs) may increase local angiotensin-converting enzyme 2 (*ACE2*) expression. ***Methods:*** In this study, we evaluated the effect of ACEi or ARB treatment on expression of *ACE2*, *ACE*, and *AGTR1* in 3-month protocol kidney allograft biopsies of stable patients using RT-qPCR ($n = 48$). Protein ACE2 expression was assessed using immunohistochemistry from paraffin sections. ***Results:*** The therapy with RAAS blockers was not associated with increased *ACE2, ACE*, or *ATGR1* expression in kidney allografts and also ACE2 protein immunohistochemistry did not reveal differences among groups. ***Conclusions:*** ACEis or ARBs in kidney transplant recipients do not affect local ACE2 expression. This observation supports long-term RAAS treatment in kidney transplant recipients, despite acute complications such as COVID-19 where ACE2 serves as the entry protein for infection.

Introduction

SARS-CoV-2 enters their target cells through the interaction with angiotensin-converting enzyme 2 (ACE2) and TMPRSS2 type 2 (transmembrane serine protease) [1]. *ACE2* expression is nearly ubiquitous; ACE2 protein was detected in endothelial cells as well as in alveolar epithelial cells in the lungs, in brush border of renal proximal tubular cells and enterocytes, and in different types of immunocompetent cells [2]. The local *ACE2* expression seems to be a requirement for organ-specific complications of a severe course of COVID-19 [3], and a highly variable clinical course of COVID-19 among similar patients' cohorts suggests different *ACE2* local expressions affected by so far unknown mechanisms. Therefore, identification of possible risk factors related to COVID-19, particularly in more vulnerable patients, is of utmost clinical relevance.

Previous preclinical studies, recently reviewed by Kreutz et al. [4], suggested that pharmacological inhibition of the renin-angiotensin-aldosterone system (RAAS) by ACE inhibitors (ACEis) or angiotensin II receptor blockers (ARBs) increases the *ACE2* expression. There are only 2 studies addressing this issue in humans. Vuille-dit-Bille et al. [5] reported the *ACE2* mRNA upregulation

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Table 1. Demographic and clinical characteristics of studied cohort

	no RAASi (n = 19)	ACEi (n = 13)	ARBs (n = 16)	p value
Recipient age, years	60 (28, 71)	46 (37, 62)	51 (31, 71)	0.266
Recipient gender, male, n (%)	15 (79)	10 (77)	12 (75)	0.962
Recipient BMI	25.7 (21, 35)	26.2 (18, 34)	28.2 (22, 39)	0.407
Retransplantation, n (%)	0	2 (15)	0	0.060
Type of donor, deceased, n (%)	19 (100)	13 (100)	16 (100)	1.000
Donor age, years	63 (26, 79)	60 (29, 66)	50 (23, 67)	0.074
Donor gender, male, n (%)	9 (47)	10 (77)	7 (44)	0.152
Dialysis vintage, months	27 (5, 47)	26 (0, 92)	20 (12, 140)	0.785
HLA mismatch	3 (1, 6)	3 (1, 4)	3 (2, 5)	0.787
Peak PRA	6 (0, 86)	8 (0, 78)	28 (0, 92)	0.197
Cold ischemia, h	15 (3, 21)	16 (2, 22)	15 (3, 20)	0.822
Original disease, n (%)				
Diabetes	2 (11)	2 (15)	2 (13)	0.678
Hypertension	2 (11)	3 (23)	0	
Glomerulonephritis	10 (53)	4 (31)	7 (44)	
Polycystic kidney disease	2 (11)	1 (8)	2 (13)	
Other	3 (16)	3 (23)	5 (31)	
T-cell depletive induction treatment	10 (53)	9 (69)	13 (81)	0.197
Maintenance immunosuppression at sampling, n (%)				
TAC/MMF/steroids	16 (84)	11 (85)	16 (100)	0.502
CyA/MMF/steroids	1 (5)	0	0	
TAC/everolimus/steroids	1 (5)	0	0	
MMF/steroids	1 (5)	2 (15)	0	
Renal function (CKD-EPI [mL/s])	0.67 [0.24; 1.34]	0.68 [0.44; 1.05]	0.74 [0.21; 0.93]	0.578
Blood pressure at sampling >140/90, n (%)	9 (47)	5 (39)	7 (44)	0.883

Continuous variables were compared using the Kruskal-Wallis test and categorical data using Fisher's exact test. ACEi, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers; CyA, cyclosporine A; MMF, mycophenolate mofetil; PRA, panel reactive antibodies; RAAS, renin-angiotensin-aldosterone system; TAC, tacrolimus.

after ACEi treatment in the duodenal epithelium. A very recently published study performed on 2 independent large cohorts of patients with heart failure showed that the use of neither ACE inhibitors nor ARBs was associated with higher plasma ACE2 concentrations [6].

Transplanted patients represent a particularly endangered subpopulation [7]. Mortality with SARS-CoV-2 infection in kidney transplant recipients is 17% (n = 115, European transplant centers, the ERA-EDTA COVID-19 Database for patients on kidney replacement therapy), which is substantially higher compared with the general population (1.7 in Spain and France –4.9% in Italy [8]) probably due to compromised immunity caused by the immunosuppressive therapy. Besides higher mortality, kidney recipients show severe symptoms in 46% of infected patients [9] compared to 19% in the general population. Therefore, identification of possible risk factors related to COVID-19 in this subpopulation is of utmost clinical relevance. As local *ACE2* transcription belongs to known risk factors of SARS-CoV-2, we aimed to evaluate the effect of RAAS inhibitors on the expression of *ACE2* and other components of RAAS (*ACE* and *AGTR1*) in kidney allografts.

Material and Methods

Kidney tissues analyzed in this study were obtained from the Transplant Laboratory Biobank in which part of biopsy samples is stored in RNA later for future transcriptomic analysis. Samples were obtained 3 months after kidney transplantation at the time of protocol biopsy from patients free of RAAS inhibitors or treated with either ARBs or ACEis. A detailed characteristic of patients is given in Table 1. Only patients without signs of acute rejection were included.

RNA was isolated from renal biopsies using the RNeasy Micro Kit (Qiagen, Hilden, Germany) and transcribed to cDNA using SuperScript™ reverse transcriptase (ThermoFisher Scientific). Gene expression profiles of *ACE* (Hs00174179_m1), *AGTR1* (Hs01096941_m1), and *ACE2* (Hs01085333_m1) were measured by RT-qPCR in triplicate for each sample, as described elsewhere [10]. RT-qPCR data were quantified using SDS 2.4 software package (Applied Biosystems), while relative gene expression values were determined using a comparative $2^{-\Delta\Delta Ct}$ method on Relative Quantification Manager Software v 1.2.1 (Applied Biosystems) with normalization to the endogenous control (*GAPDH* and Hs999999905_m1). As a calibrator, 1 of the samples from control group was used. Statistical analyses were performed using SPSS v.20.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad InStat v.3.05 for Windows (GraphPad software, San Diego, CA, USA).

ACE-2 protein was detected in formalin-fixed, paraffin-embedded 4-µm tissue sections of kidney allograft biopsies using mouse anti-human ACE-2 monoclonal antibody (MAB933) at 10 µg/mL. IHC was performed using a Ventana BenchMark Ultra automated IHC stainer (Ventana Medical Systems, Roche Diagnostics). Epitope retrieval was performed onboard, using Ventana Cell Conditioning 1 (950–224) solution for 64 min at 95°C, and primary antibody was then incubated for 32 min at 37°C. As a visualization system, a Ventana OptiView DAB IHC Detection Kit (760–700) was used, and nuclei were (on board) counterstained with hematoxylin. The ACE-2-stained area was calculated using Fiji ImageJ software (https://fiji.sc/) [11] as a ratio of the IHC-stained area to the total cortex area after exclusion of glomeruli and arteries.

Results

For the purpose of this study, clinical data and biobank-stored kidney allograft samples from 48 patients who had undergone deceased donor kidney transplantation in 2013–2019 and protocol biopsy at 3 months were evaluated. The primary aim of the study was to evaluate association of ACEis and ARBs with intrarenal *ACE2, ACE,* or *AGTR1* gene expressions. Therefore, 3 original groups were retrospectively formed: ACEis (n = 13), ARBs (n = 16), and patients free of RAAS inhibitors (n = 19). No differences were found in main demographic and pretransplant clinical characteristics among treatment groups (Table 1). Also, kidney allograft function at the time of 3-month protocol biopsy was similar (p = 0.578). Immunosuppression at sampling was almost similar among groups (p = 0.502). The expression of *ACE2, ACE*, or *AGTR1* was not significantly affected by T-cell depletive treatment (p = 0.213, 0.861, and 0.134, respectively). The concomitant therapy with RAAS blockers was not associated with increased *ACE2, ACE*, or *ATGR1* expression in kidney allografts (Fig. 1a–c), albeit there was significant individual variability.

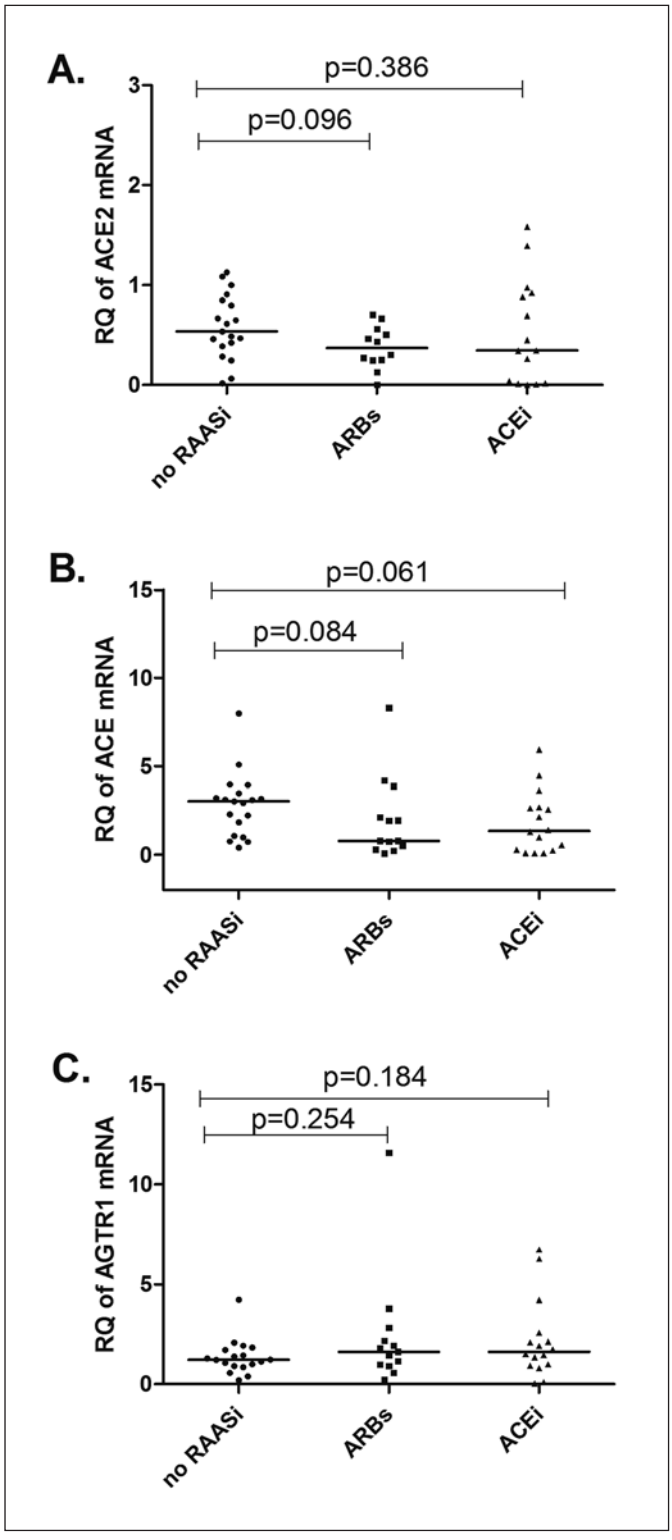
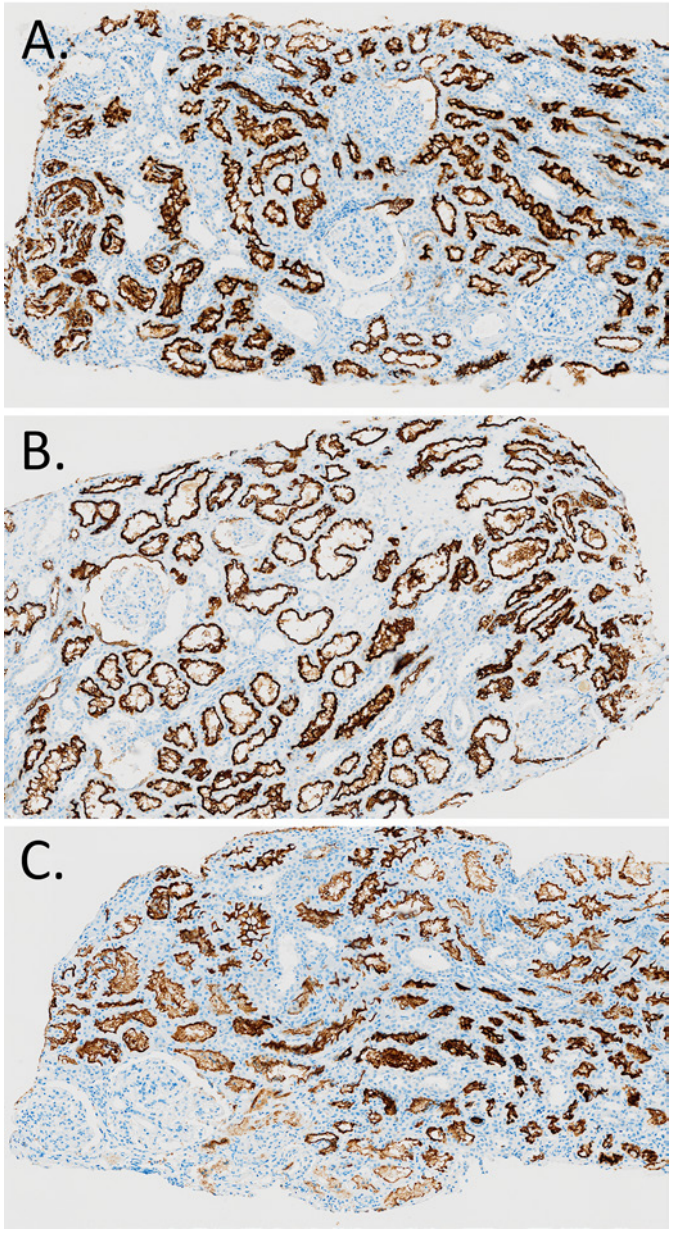


Fig. 1. Expression of *ACE* (**A**), *AGTR1* (**B**), and *ACE2* (**C**) mRNA in kidney allografts is not affected by RAAS inhibitors (no RAASi, no treatment; ARBs; ACEi). RQ, relative quantity (fold change related to calibrator); ARB, angiotensin II receptor blocker; ACEi, ACE inhibitor; ACE2, angiotensin-converting enzyme 2.



Color version available online

Fig. 2. A Representative example of ACE2 immunohistochemical staining (brown) in kidney allografts of patients treated with no RAAS inhibitors (**B**), ACEi (**C**), and ARBs. ACEi, ACE inhibitor; ARB, angiotensin II receptor blocker; ACE2, angiotensin-converting enzyme 2.

Next, we aimed to examine ACE2 expression at the protein level. As shown in Figure 2, there is no difference in ACE2 protein abundance in kidney graft biopsies among groups. This observation was confirmed by calculating a ratio of the IHC-stained area to the total cortex area after exclusion of glomeruli and arteries, and no differences were found between treatment groups (p = 0.762). Although parietal cells of the Bowman capsule were positive for ACE2 in some glomeruli, their semi-quantitative evaluation showed no difference among groups.

Discussion

In the present study, we show the unique data concerning the *ACE2* mRNA local expression in kidney allografts in patients with different antihypertensive treatment. This report does not prove any effect of RAAS blockers, ACEis, or ARBs, on local *ACE2* mRNA transcripts, which was hypothesized. This observation is in line with recent clinical report from 2 independent heart failure cohorts where the treatment by neither ACE inhibitors nor ARBs did not affect plasma ACE2 concentrations [6]. Our findings also support recent epidemiological studies [12, 13] where no effect of RAAS inhibitors on COVID-19 outcomes was noticed.

Clearly, direct local tissue ACE2 measurement is the way to prove the effect of concomitant therapy on its expression. However, it is difficult to obtain native kidney biopsy material as it has not been routinely performed in patients suffering from COVID-19 disease. In contrary, in kidney transplantation, the protocol biopsy, performed as early as at 3 months in patients without graft rejection, may give the opportunity to study renal tissue from donors of different age and comorbidities. Histological assessment of 3-month protocol biopsies may serve as reflection of donor histology, and thus, our data are likely to have general reproducibility aside from transplant population.

The strength of our study is the direct determination of *ACE2* mRNA expression in rarely accessible human tissue, that is, kidney allograft, in well-defined cohort of patients. Nevertheless, there are some limitations as well. First, it is relatively a low number of subjects in cohorts. Second, the data were obtained from patients who underwent kidney transplantation and were under immunosuppressive treatment which may modify *ACE2* transcripts. On the other hand, the immunosuppressant therapy was similar across all 3 groups, and therefore, it does not introduce any specific bias. In conclusion, our data support the hypothesis that ACEi or ARB treatment does not represent any additional risk for unfavorable COVID-19 disease as they have no effect on local COVID-19 entry protein ACE2 expression.

Statement of Ethics

Biopsies were obtained by biobanking program approved by the review boards of the Institute for Clinical and Experimental Medicine in Prague under A 13-02-01 (83/13). All patients participated in this study signed informed consent.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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Author Contributions

Monika Cahova participated in research design, data analysis, and writing the paper; Martin Kveton performed research and participated in data analysis; Vojtech Petr participated in data analysis; David Funda performed the research; Helena Dankova performed the research; Ondrej Viklicky participated in research design, data analysis, and writing the paper; and Petra Hruba participated in research design, performed the research and data analysis, and participated in writing the paper.

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