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Serum HSP27 is associated with medullary perfusion in kidney allografts

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Abstract

Background—Heat shock protein 27 (HSP27) is a small HSP up-regulated in response to stress in the kidney. The relationship between HSP27 and intrarenal oxygenation in patients with native and transplant kidney disease is unknown.

Methods—We compared HSP27 levels, intrarenal oxygenation measured by blood oxygen-level dependent (BOLD) imaging using R_2^* values, and perfusion determined by arterial spin labeling (ASL) magnetic resonance imaging (MRI), between patients with native and transplant kidney disease (n=28).

Results—There were no statistical differences in mean age (53.9 vs. 47.1 years), kidney function (63.6 vs. 50.7 ml/min per 1.73 m²), mean arterial blood pressure (91.6 vs. 91.1 mm Hg), hematocrit (40.6% vs. 39.3%), diuretic or angiotensin-converting enzyme inhibitor use, serum or urine levels of hydrogen peroxide, nitric oxide, F₂ isoprostanes and HSP27 between native and transplant kidneys. BOLD-MRI studies demonstrated comparable patterns in intrarenal oxygen bioavailability (medullary R_2^* 18.1 vs. 18.3/s and cortical R_2^* 12 vs. 11.7/s, respectively). However, medullary perfusion was significantly lower in transplant kidneys (36.4 vs. 78.7 ml/100 g per minute, p=0.0002). There was a linear relationship between serum HSP27 concentrations and medullary perfusion in kidney allografts (HSP27 concentration [ng/mL] = 0.78 + 0.09 medullary perfusion, $R^2=0.43$, p=0.01).

Conclusions—Our study demonstrates that medullary perfusion is significantly lower in kidney allografts compared with native kidneys with comparable renal function. We further noted a direct association between serum HSP27 levels and medullary perfusion after transplantation. Additional studies are needed to examine the role of HSP27 as a biomarker of kidney disease progression.

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Keywords

BOLD; HSP27; Kidney transplantation; Oxidative stress; Perfusion

Introduction

The development of progressive kidney allograft dysfunction is associated with immunological mechanisms, calcineurin inhibitor toxicity, polyomavirus and other factors (1–3). These factors can promote the development of an imbalance between prooxidants and antioxidants (4). Oxidative stress plays a major role in inflammation, apoptosis and fibrosis that can lead to interstitial fibrosis and tubular atrophy (IF/TA), the histopathological end points associated with chronic allograft failure (5, 6).

Another component of kidney allograft dysfunction and chronic kidney disease (CKD) is the presence of chronic hypoperfusion and hypoxia, which increase intrarenal oxidative stress (7–9). Noninvasive radiological methods to evaluate renal perfusion and oxygenation include functional magnetic resonance imaging (fMRI), which allows the evaluation of kidney perfusion and obtains anatomical and functional information simultaneously (7, 10).

Heat shock proteins (HSP) constitute a protein superfamily that responds to heat and other physiological stresses (11, 12). HSP27, or HSP25 in mice, belongs to the small HSP subfamily, is strongly induced by oxidative stress, and has cytoprotective effects through regulation of the actin cytoskeleton as well as inhibition of oxidative injury and apoptosis (13–16). High levels of HSP27 are normally present in the renal medulla (17), a region of the kidney exposed to severe hypoxia (7, 18) and osmotic stress (17, 19), suggesting a protective role for this protein against these types of stress (20–22). We hypothesized that HSP27 is involved in the regulation of intrarenal oxygenation and that serum HSP27 level is a biomarker of renal perfusion. To test this hypothesis we compared 2 groups of patients: transplant patients with chronic allograft dysfunction and patients with native chronic kidney disease.

Subjects and methods

Patients

Kidney transplant recipients and subjects with CKD were recruited by referring nephrologists, in consecutive order, when they presented to their routine clinic appointments, if they met the study's inclusion criteria. Subjects were included in the study if they were adults (>18 years old), MRI compatible and clinically stable between visits. Subjects were considered stable if their serum creatinine levels varied ≤ 0.3 mg/dL between visits, and no events changed their clinical status during the interim. Subjects were included in the study regardless of their underlying disease process. Serum creatinine levels and estimated glomerular filtration rate (eGFR) were used to ensure we recruited subjects with a wide range of renal function (23). Transplant patients were at least 6 months posttransplantation. Patients were recruited between December 2007 and February 2009. All patients underwent blood oxygen level dependent (BOLD) and perfusion MR imaging.

Blood samples were collected on the same day the MRI was performed. Blood samples were assayed for standard chemistries, complete blood count and biomarkers of oxidative stress. Urine samples were assayed for oxidative stress, chemistries and urinalysis. Research involving human subjects was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained in writing from all subjects, and the study was carried out in compliance with a study protocol reviewed and approved by the University of Wisconsin Institutional Review Board.

BOLD and perfusion MRI

MRI scans were performed on a 1.5-tesla MR scanner (Signa HDx; GE Healthcare, Milwaukee, WI, USA) with an 8-element phased array cardiac coil (GE Healthcare, Milwaukee, WI, USA). BOLD imaging was performed using a multiecho gradient recalled echo (GRE) sequence with 16 echoes at 1-mm intervals and 5-mm slice thickness prescribed in the coronal plane. The scanning parameters were as follows: TR = 87 milliseconds, TE = 8 – 41.8 milliseconds, flip = 40°, BW = 62.5, FOV = 32–34 cm, matrix = 256×128, NEX = 1. Each set of 16 T2*-weighted images was acquired during an 11-second breath hold. BOLD MR data were processed using GE Functool; color R2* maps were generated and regions of interest (ROI) were placed in the medulla and cortex to obtain mean cortical and medullary R2* values (1/s).

Perfusion imaging using arterial spin labeling (ASL) was acquired using a respiratory triggered flow-sensitive alternative inversion recovery (FAIR)-balanced steady state free precession (b-SSFP) acquisition with the following readout parameters: TR/TE/flip = 4.6/2.3 ms/70°, BW = 83.33 kHz, FOV = 34–36 cm, and 128×128 matrix. The 8-mm imaging slice, carefully chosen not to include the feeding vessels, was central to a 20-mm slice selective inversion thickness. Proton density images (to measure M₀) were obtained with a NEX = 4 using the b-SSFP readout with no inversion preceding it. Perfusion data were analyzed using custom scripts written in MATLAB (version 7.5; The MathWorks Inc., Cambridge, MA, USA), using automated rigid registration based on normalized mutual information and a 1-compartment model for perfusion calculation. Flow values were calculated on a pixel-by-pixel basis in the cortex and medulla separately and then averaged together.

Biomarkers of oxidative stress

To reduce assay variability, each sample was measured in triplicate, and the experiment was repeated 3 times for each of the biomarkers assessed, as described previously (5, 6). We used an ELISA Kit from Stressgen Biotechnologies (cat. no. EKS-500; Victoria, BC, Canada) to measure HSP27, according to the manufacturer's recommendations. Total nitric oxide (NO) levels (nitrate/nitrite) were measured using the NO (total) Detection Kit from Stressgen Biotechnologies (cat. no. EKS-310; Victoria, BC, Canada) according to the manufacturer's recommendations. Serum and urine H₂O₂ levels were measured using the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit from Molecular Probes, Invitrogen Corporation (cat. no. A-22188; Carlsbad, CA, USA) according to the manufacturer's recommendations. Serum and urine 8-iso prostaglandin levels were measured using the

ELISA Kit from Stressgen Biotechnologies (cat. no. EKS-200; Victoria, BC, Canada) according to the manufacturer's recommendations.

Statistical analysis

Parametric and nonparametric numerical data are expressed as means (SD) or medians (range). Numerical data between the groups were compared using Student's *t*-test (parametric test) or the Mann-Whitney test (nonparametric test) depending on whether the data had a normal or non-normal distribution by Shapiro-Wilks test. Evaluation of nominal data between groups was performed using chisquare test analysis or Fisher's exact test. Multiple linear regression and correlation tests (Pearson correlation coefficient or Spearman rank correlation for parametric and nonparametric data, respectively) were performed using Graph Pad Prism 5 software and MedCalc Statistical Software. A 2-tailed *p* value ≤ 0.05 , not adjusted to account for multiple testing, was considered significant.

Results

Patient characteristics

We studied 14 patients with native and 14 patients with transplant CKD (CKD-T), both groups with stable kidney function. Baseline characteristics are summarized in Table I. There were no statistically significant differences in demographic, clinical and biochemical characteristics between native kidney and transplant groups. Kidney function was comparable in CKD and CKD-T groups (serum creatinine 1.1 ± 0.8 vs. 1.8 ± 0.1 mg/dL, $p=0.1$; eGFR 63.6 ± 25 vs. 50.7 ± 18.7 ml/min, $p=0.09$). Furthermore, there were no differences in pharmacological treatments, except for the use of immunosuppressants in the transplant group. However, we realize that the lack of statistical significance cannot be taken as a reliable proof that these differences are not due to confounding variables.

BOLD and ASL MR results

There were no significant differences in medullary (18.1 ± 2.6 vs. 18.5 ± 1.3 1/s) and cortical (12 ± 0.7 vs. 11.7 ± 0.7 1/s) oxygen bioavailability between patients with native and transplant kidney disease (Tab. II). The medullary to cortical oxygenation ratio was also similar between the 2 groups (1.5 ± 0.2 vs. 1.5 ± 0.1). Notably, kidney allografts showed significantly lower medullary perfusion values (36.4 ± 19.4 vs. 78.7 ± 24.3 ml/100 g per minute, $p=0.0002$) and lower cortical perfusion (277.4 ± 86.1 vs. 337.5 ± 126.6 , ml/100 g per minute, $p=0.1$) than native kidneys.

Oxidative stress and serum HSP27

We found no statistically significant differences in HSP27 levels between transplant patients and the group with native CKD (4.2 ± 0.7 ng/mL vs. 2.9 ± 1.5 ng/mL, $p=0.1$). Serum and urine levels of NO, hydrogen peroxide and F₂ isoprostanes were comparable between both groups, yet transplant recipients showed a trend to a higher global prooxidant milieu (Tab. III).

Notably, we observed a statistically significant relationship between serum HSP27 concentration and medullary perfusion in transplant patients (Fig. 1). Regression analysis

yielded a linear equation ($\text{Serum HSP27} = 0.7873 + 0.09366 \times \text{Medullary Perfusion}$, $R^2=0.43$, $p=0.01$), making it possible to predict medullary perfusion from HSP27 serum levels and vice versa. After log transformation we found the same R^2 and a p value of 0.006 instead of 0.01. Stepwise Cox regression analyses adjusting for age, sex and eGFR, retained serum HSP27 as an independent predictor of medullary perfusion ($\text{Medullary Perfusion} = 16.98 + 4.62 \times \text{Serum HSP27}$, $R^2=0.43$, $p=0.01$).

Discussion

Our study demonstrates that systemic oxidative stress is similar in patients with CKD and CKD-T with similar renal function, that renal perfusion is lower in kidney allografts compared with native kidneys and that serum HSP27 has an association with medullary perfusion in kidney allografts. However, our study is limited by a small sample size and heterogeneous data.

In healthy subjects, medullary perfusion is lower than perfusion in the cortex due to the redistribution of blood flow toward the cortex to optimize glomerular filtration and reabsorption, high workload of the tubular component and preservation of the osmotic gradient by active reabsorption of sodium (24). These observations are confirmed by the administration of furosemide, a loop diuretic that reduces the reabsorptive workload in the ascending limb and leads to medullary oxygenation levels that approach those in the cortex (25). In the present study we found lower whole kidney perfusion rates in transplant patients than in patients with native kidney disease measured with a FAIR-ASL technique. The differences in medullary perfusion were statistically significant. It is likely that in situ inflammation, vasoconstrictive effects of calcineurin inhibitors, the anatomic position of the allograft and arterial anastomosis explain this finding.

HSP27 belongs to the superfamily of small HSPs that are synthesized in response to several stress factors such as hypoxia and oxidative stress, and have a cytoprotective role. In healthy kidneys, HSP27 is present at the highest levels in the medulla (21), possibly in response to conditions of hypoxia (10, 22) and osmotic stress (21, 23). We found reduced medullary HSP27 mRNA and HSP27 protein levels in rat kidney allografts with chronic allograft nephropathy (CAN) compared with syngeneic transplants (26). In contrast, HSP27 was increased in the cortex of kidney allografts with CAN or acute rejection, suggesting a role for this protein as a stress-response molecule during acute and chronic rejection. Since chronic rejection is a profibrotic process, we examined the role of HSP27 in epithelial-to-mesenchymal transition (EMT) of rat tubular epithelial cells and observed a significant increase in its mRNA and protein levels in response to TGF- β 1 (24). HSP27 colocalized with E-cadherin and F-actin in cells undergoing EMT and increased E-cadherin levels too. These observations suggest that HSP27 may modulate EMT through up-regulation of E-cadherin (20). HSP27 may also be used as a biomarker of kidney disease. Consistent with this hypothesis, we observed greater serum HSP27 levels in kidney transplant recipients with CAN compared with healthy volunteers (5, 6).

In the current study, we measured serum and urine HSP27 levels in patients with CKD and CKD-T with comparable kidney function. We found no significant differences in HSP27

levels but observed an association between serum HSP27 and medullary perfusion. Since HSP27 is induced by stress, it is possible that decreased blood flow stimulates the mitogen-activated protein kinase pathway, which in turn activates HSP27 to regulate intrarenal oxygenation. Indeed, despite lower medullary perfusion in kidney allografts, there was no significant difference in oxygen bioavailability between the 2 groups. This suggests regulatory mechanisms that maintain medullary oxygenation independent from perfusion. High levels of HSP27 in the renal medulla reported previously by our group support this hypothesis (17). However, mechanistic studies are needed to demonstrate the role of HSP27 as a modulator of medullary oxygenation.

This is mainly a negative study limited by low statistical power due to the small sample size compared with the high variability of the biomarkers. However, our findings suggest that kidney function plays an important role in the regulation of oxidative stress in native and transplant kidney disease, and support further studies to examine the role of HSP27 as a biomarker of kidney disease.

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Fig. 1. Regression analysis indicated a significant linear relationship between serum HSP27 concentrations and medullary perfusion rates corresponding to the equation shown and represented by the solid black line.

TABLE I

BASELINE CHARACTERISTICS

	Native kidney	Transplant	p Value
Number	14	14	N/A
Age, years	53.9 (12.9)	47.1 (13.5)	0.2
Sex, % female	50	21	0.3
Weight, lb	164.8 (34.9)	184.8 (38.3)	0.2
MAP, mm Hg	91.6 (10.6)	91.1 (8.5)	0.9
Diabetic patients, %	7.1%	14.3%	0.6
Serum creatinine, mg/dL	1.1 (0.7 – 3.4)	1.3 (1 – 4)	0.1
eGFR, ml/min per 1.73 m ²	63.6 (25.9)	50.7 (18.7)	0.09
Htc, %	40.6 (4)	39.3 (4.2)	0.4
Presence of proteinuria, %	14.29	28.57	0.7
Urine protein, mg/dL	110.4 (0–1,384.9)	32.7 (5–156.2)	0.2
Urine Na, mmol/L	81.3 (35.3)	95.9 (47.4)	0.4
Urine, Osm	511.9 (251.9)	503.8 (176.8)	0.6
Diuretic, %	14.3	7.1	1
ACEI/ARB, %	21.4	35.7	0.7
Statin, %	21.4	57.1	0.1
CNI, %		71.4	N/A
TAC, ng/mL (n=6)		6.5 (2.5)	N/A
CsA, ng/mL (n=3)		87 (14)	N/A
MRI, time after Tx, months		82.9 (29.5)	

Values are means (SD) or medians (range), unless indicated otherwise.

MAP = mean arterial pressure; eGFR = estimated glomerular filtration rate; Htc = hematocrit; ACEI/ARB = angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; CNI = calcineurin Inhibitors; TAC = tacrolimus; CsA = cyclosporine; MRI = magnetic resonance imaging; Tx = kidney transplant.

TABLE II

PERFUSION MRI AND BOLD-MRI VALUES

	Native	Transplant	p Value
MR2*, 1/s	18.1 (2.6)	18.3 (1.3)	0.5
CR2*, 1/s	12 (0.7)	11.7 (0.7)	0.3
MCR2*	1.5 (0.3)	1.5 (0.2)	0.8
Cortical perfusion, ml/100 g per minute	337.5 (126.6)	277.4 (86.1)	0.1
Medullary perfusion, ml/100 g per minute	78.7 (24.3)	36.4 (19.4)	0.0002

Values are means (SD).

MRI = magnetic resonance imaging; BOLD-MRI = blood oxygen-level dependent MRI; MR2* = medulla R2*; CR2* = cortical R2*; MCR2* = index medulla/cortex R2*.

TABLE III**OXIDATIVE STRESS BIOMARKERS IN PATIENTS WITH NATIVE OR TRANSPLANT KIDNEYS**

	Native	Transplant	p Value
HSP27 serum, ng/mL	2.9 (0.4)	4.2 (0.7)	0.1
HSP27 urine, ng/mL	1.3 (0.6)	0.8 (0.3)	0.1
NO serum, mmol/L	56.2 (4.8)	79.7 (11)	0.08
NO urine, mmol/L	758.6 (225)	598.3 (107)	0.9
H ₂ O ₂ serum, mM	3.6 (1)	11.4 (3.8)	0.1
H ₂ O ₂ urine, mM	5.1 (1.1)	8.3 (1.3)	0.08
F ₂ isoprostanes urine, pg/mL	12,116 (3,335)	19,854 (5,116)	0.4

Values are means (SD) or p values.

NO = nitric oxide.