



The Role of NF- κ B in the Downregulation of Organic Cation Transporter 2 Expression and Renal Cation Secretion in Kidney Disease

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Organic cation transporter 2 (OCT2), encoded by the *SLC22A2* gene, is the main cation transporter on the basolateral membrane of proximal tubular cells. OCT2 facilitates the entry step of the vectorial transport of most cations from the peritubular space into the urine. OCT2 downregulation in kidney disease models is apparent, yet not clear from a mechanistic vantage point. The aim of this study was to explore the role of inflammation, a common thread in kidney disease, and NF- κ B in OCT2 modulation and tubular secretion. Among the OCTs, OCT2 was found consistently downregulated in the kidney of rats with chronic kidney disease (CKD) or acute kidney injury (AKI) and in patients diagnosed with CKD, and it was associated with the upregulation of TNF α renal expression. Exposure to TNF α reduced the expression and function of OCT2 in primary renal proximal tubule epithelial cells (RPTEC). Silencing or pharmacological inhibition of NF- κ B rescued the expression of OCT2 in the presence of TNF α , indicating that OCT2 repression was NF- κ B-dependent. *In silico* prediction coupled to gene reporter assay demonstrated the presence of at least one functional NF- κ B *cis*-element upstream the transcription starting site of the *SLC22A2* gene. Acute inflammation triggered by lipopolysaccharide injection induced TNF α expression and the downregulation of OCT2 in rat kidney. The inflammation did reduce the active secretion of the cation Rhodamine 123, with no impairment of the glomerular filtration. In conclusion, the NF- κ B pathway plays a major role in the transcriptional regulation of OCT2 and, in turn, in the overall renal secretory capacity.

Keywords: chronic kidney disease, inflammation, NF- κ B, organic cation transporter, TNF- α , tubular secretion

INTRODUCTION

Solutes are excreted from the body via the kidneys through three concerted processes: glomerular filtration, tubular secretion and reabsorption (1–3). For decades, glomerular filtration was considered the predominant process in renal excretion, yet from an evolutionary standpoint, tubular secretion appears fundamental, with glomerular filtration evolving only afterwards (4). Only later, tubular secretion was demonstrated to be critical for the clearance of organic ions and even involved in the elimination of salt and water (5, 6). Tubular secretion occurs, for the most part, at the proximal tubule level, and, as demonstrated in stop-flow and micropuncture seminal studies in mammalian, fish, and avian kidneys, it seems particularly relevant for positively charged solutes including endogenous substances, xenobiotics and drugs, the latter accounting for close to 40% of approved compounds with marketing authorization by regulatory agencies (7). The first step of cation tubular secretion, the translocation of the substrate from the interstitial space into the proximal tubular cell, is often rate-limiting and it is primarily mediated by the organic cation transporters 1, 2 and 3 (OCT1–3), Na⁺-independent, polyspecific transporters expressed on the basolateral membrane of proximal tubular cells (8). Extensive pharmacogenetic evidence indicates that among the OCTs, OCT2 is the primary route of renal elimination of several drugs to the point that guidance of drug regulatory agencies demands that each molecule in development be tested *in vitro* for inhibition of OCT2 transport activity in order to predict potential drug-drug interactions (9, 10).

It has been recently shown that, among patients with end-stage kidney disease, protein-bound substances, which are prominently eliminated by active tubular secretion, accumulate in substantially higher concentrations compared to free solutes that are mainly filtered (11). Serum creatinine, which is the staple marker of kidney function, is a substrate of OCT2, which may be accountable for up to 30% of the overall creatinine clearance (12). Likewise, Trimethylamine-N-oxide (TMAO) level, an OCT2 substrate (13), was found markedly higher in the plasma of patients with chronic kidney disease receiving dialysis than in the plasma of healthy individuals. Moreover, renal transplantation resulted in substantial reductions in TMAO plasma concentration (14), suggesting that OCT2 activity might be somewhat reduced in patients with CKD. Indeed, the renal OCT2 expression seems to be highly affected by the underlying pathological context. Different animal models of kidney damage were characterized by a lower expression of Oct1 and Oct2 and a reduced clearance of OCT substrates such as cimetidine, famotidine and tetraethylammonium (TEA) as compared with the control animals (3, 15–17). Conversely, mice fed a high-fat diet with reduced kidney function were characterized by a higher expression of Octs in comparison with lean animals (18). The modulation of OCT expression in kidney disease is likely of pleiotropic nature and may involve alteration in various protein kinase activity and activation of the ischemia/reperfusion-inducible protein (IRIP) (19–22). The recently published results of the Chronic Renal Insufficiency Cohort (CRIC) study on 3,939 participants revealed that biomarkers of inflammation inversely

TABLE 1 | Clinical and demographic data of patients.

	Normal (n = 10)	CKD (n = 5)	P-value
Age (years)	53.9 ± 4.40	46.8 ± 6.83	0.038
Gender (M/F)	6/4	1/4	0.282
Serum Creatinine (μ mol/L)	59.1 ± 4.24	132.8 ± 5.85	0.0007
eGFR (mL/min/1.73 m ²)	102 ± 8.93	43.8 ± 4.55	0.0007
Category (%)			
G1	10 (100)	0 (0)	
G2	0 (0)	0 (0)	
G3a	0 (0)	3 (60)	
G3b	0 (0)	2 (40)	
G4	0 (0)	0 (0)	
G5	0 (0)	0 (0)	

Data are expressed as the mean \pm SD. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; M, male; F, female; sCr, serum creatinine. eGFR was calculated with the formula: = $141 \times \min(\text{sCr}/\kappa, 1)^{\alpha} \times \max(\text{sCr}/\kappa, 1)^{-1.209} \times 0.993^{\text{age}} \times 1.018$ [if female] $\times 1.159$ [if Black]. $\kappa = 0.7$ (females) or 0.9 (males). $\alpha = -0.329$ (females) or -0.411 (males). Min, indicates the minimum of sCr/ κ or 1; Max, indicates the maximum of sCr/ κ or 1.

correlated with the measures of kidney function and positively with albuminuria (23). In the present study, we investigated the role of local inflammatory state, a common thread in the evolution of diseases with distinct etiopathogenesis different such as CKD and AKI (24), and its main transcriptional mediator NF- κ B, in the regulation of the expression of OCTs and the pathological and pharmacological ramifications thereof.

MATERIALS AND METHODS

Patient Characteristics

Diagnosis of CKD was made according to the residual renal function assessed per serum creatinine level and the Kidney Disease Improving Global Outcomes (KDIGO) classification (Table 1). Renal biopsies were obtained from five patients with chronic kidney disease (CKD). The patient was placed in the prone position; the puncture point was marked at the level of the lower kidney. Under local anesthesia, the needle was inserted into the renal capsule under ultrasound guidance, and the renal cortex tissue was ejected from the puncture needle. Pathologically normal samples were isolated from the tissue surrounding the tumor removed from ten patients who underwent partial nephrectomy at the Liaocheng University/Liaocheng People's Hospital between December 2019 and December 2020.

Animal Housing and Generation of the Experimental Models

Only male rats were used in this study because the expression level of OCT2 in the kidneys of male rats is higher than that in female rat kidneys (25, 26). Male Sprague-Dawley rats (200–220g) from Vital River Laboratories (Beijing, China) were placed in the animal facility with a 12:12-h day and night cycle and ad libitum food (AIN93 M, Keao Xieli feed co. LTD, Beijing China) and water. Chronic kidney disease (CKD) was induced by a two-stage procedure of 5/6 nephrectomy with lateral incision to expose the retroperitoneal kidney, as previously

described (27). All rats were sacrificed under anesthesia 4 weeks after the second stage of surgery, kidneys were excised and halved by longitudinal incision to expose the cortex, which was isolated for further analyses. Acute kidney injury (AKI) was induced by unilateral with contralateral nephrectomy, as previously described (28). Briefly, the right-sided nephrectomy or sham procedure was performed, then the left renal artery was carefully dissected and occluded for 30 min using a non-traumatic microvascular clamp. All rats were humanely sacrificed by CO₂ inhalation, 24 h after the surgery. Kidneys were harvested and halved by longitudinal incision to expose the cortex, which was isolated for further analyses. For each disease model, sham operations were carried out by performing a lateral incision without disturbing the internal organs, under the same anesthesia conditions. Inflammation was induced by daily intraperitoneal injection of 5mg/kg body weight endotoxin lipopolysaccharide (L2880, LPS from *Escherichia coli*, Sigma-Aldrich, St. Louis, MO) for 3 days. Control animals received the same dose of detoxified LPS (dLPS), uncoupled from the biologically active component lipid A, essential for the activation of inducible nitric oxide synthases (iNOS) (29). LPS and dLPS were dissolved in sterile Hank's Balanced Salt Sodium (HBSS, Gibco, UK) supplemented with 10 mM HEPES (Gibco, UK), and adjusted to pH 7.4 with NaOH. Twelve hours after the last injection, three animals per group were euthanized by CO₂ inhalation, the kidneys harvested for mRNA, and protein expression studies, two animals per group were subjected to the Rhodamine 123 excretion assay and then sacrificed by CO₂ inhalation.

Cell Culture

Human RPTEC (Human Renal Proximal Tubule Epithelial Cells) cell line was obtained from Lonza (Basel, Switzerland). Cells were grown in renal epithelial cell growth medium bullet kit (CC-3190, Lonza, Basel, Switzerland) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 μ g/ml streptomycin. For differentiation, the percentage of FBS was reduced from 10 to 2%.

Rhodamine 123 Excretion Assay

The urinary excretion of Rhodamine 123 was monitored as previously reported (30). Rats were hydrated by subcutaneous injection of 1 ml 0.9% NaCl 1 h before the initiation of the perfusion. The perfusion was performed under anesthesia with i.p. pentobarbital (60 mg/kg, Southland Pharmaceutical, Guangzhou, China). Furosemide (10 mg/kg, Southland Pharmaceutical, Guangzhou, China) was injected i.p. to prevent the deterioration of the distal nephron. Heparin (125 IU/100 g, Qianhong Bio-pharma, Changzhou, China) was injected into the spleen to reduce the risk of blood clot formation. The renal artery of the right kidney was cannulated via the mesenteric artery without interruption of the blood flow, and a cannula was inserted into the ureter. The excised kidney was connected to a recirculation perfusion system, which was oxygenated with a 95% O₂/5% CO₂ mixture and placed in a fluid bath with a constant temperature of 37.5°C. The perfusion fluid consisted of: 112.0 mM NaCl, 5.2 mM KCl, 2.0 mM CaCl₂, 1.0 mM MgCl₂,

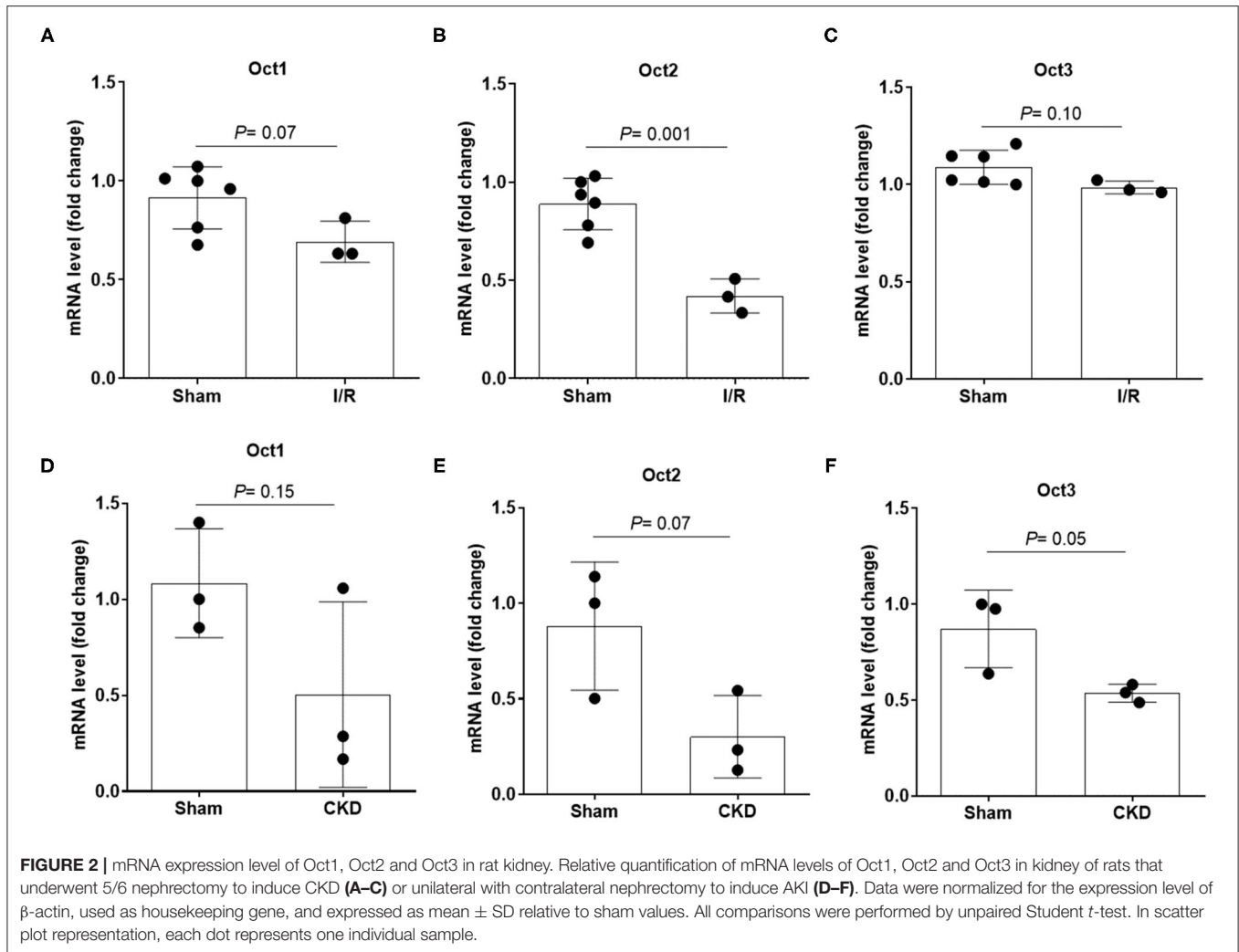
25 mM NaHCO₃, 0.84 mM Na₂HPO₄, 0.28 mM KH₂PO₄, 5.0 mM D-glucose, 4.0 mM urea, 25.0 g/l pluronic F108, 0.33 mM glutathione, 0.083 mM Myo-inositol, 0.50 mM cysteine, 2.3 mM glycine, 2.0 mM Na-pyruvate, 1.22 mM Na-acetate, 0.21 mM Na-propionate, 1.0 mM inosine, 5.0 mM alanine, 0.11 mM Na-glutamate, 2.0 mM L-glutamine, 0.01 mM ascorbic acid, 1.0 mM Na-lactate, 1.0 mg/l choline chloride, 4 IU/l insulin, 2.0 μ g/l aldosterone, 10 μ g/l anti-diuretic hormone, 20.0 μ g/l andangiotensin II. To this solution, 8.5% Travasol (1B6624, Baxter, Shanghai, China) was added to a final concentration of 0.1%. After perfusion for 30 min to equilibrate the kidney, the perfusion fluid was supplemented with a solution containing Rhodamine 123 (R8004, Sigma-Aldrich, St. Louis, MO) to a final concentration of 525 nM. Urine samples were collected every 10 min. Perfusate samples were drawn at the midpoint of each urine collection interval. For the determination of the glomerular filtration rate (GFR), tetramethylrhodamine isothiocyanate-dextran (T1037, TRITC-Dextran, 11.4 μ M, 4400 mol wt, Sigma-Aldrich, St. Louis, MO, USA) was added to the perfusion fluid 10 min after the stabilization period. The concentrations of Rhodamine 123 and TRITC-dextran in urine samples were determined directly after the experiment by fluorescence spectrophotometry using a Perkin Elmer LS50B (Perkin Elmer, Beaconsfield, Buckinghamshire, UK).

Isolation of RNA From Kidney Tissue and Quantification of Transcript Levels

Total RNA was isolated using Trizol (ThermoFisher scientific, Waltham, Massachusetts, USA) according to the manufacturer's instruction. After DNase (Promega, Madison, WI, USA) treatment, two μ g of total RNA was reverse transcribed using oligo-dT primers and Superscript II (ThermoFisher scientific, Waltham, Massachusetts, USA). Complementary DNA (cDNA) was used for real-time polymerase chain reaction analysis with TaqMan master mix and primers (Applied Biosystems, CA, USA). Transcript levels, determined in two independent complementary DNA preparations, were calculated and expressed relative to the housekeeping gene b-actin. The TaqMan probes used in this study are: human OCT1, Hs00427550_m1; OCT2, Hs00533907_m1; OCT3, Hs01009568_m1; TNFa, Hs00174128_m1; iNOS: Hs01075527_m1; SGLT1: Hs01573793_m1; SGLT2: Hs00894642_m1; b-ACTIN, Hs99999903_m1; rat Oct1, Rn00562250_m1; Oct2, Rn00580893_m1; Oct3, Rn00570264; Tnfa, Rn99999017_m1, Mdr1: Rn01639253_m1; Mate1: Rn01460731_m1; b-actin, Rn00667869_m1.

Immunostaining

Human and rat kidney tissues were fixed in 4% paraformaldehyde for 1 h and then kept at 4 degree until paraffin embedding. Kidney paraffin sections (4- μ m-thick) were hydrated, microwaved for 8–15 min in 10 mM sodium citrate (pH 6.0) for antigen retrieval, and then probed with a rabbit antibody against OCT2 (1:100, LS-C357581, LSBio, Seattle, WA, RRID:AB_2891045), OCT3 (1:100, LS-C406026, LSBio, Seattle, WA, RRID:AB_2891046) or TNF α (1:100, ab6671, Abcam, Cambridge, United Kingdom, RRID:AB_305641). Immunolabeled sections were then



Construction of Human OCT2 Promoter Vectors

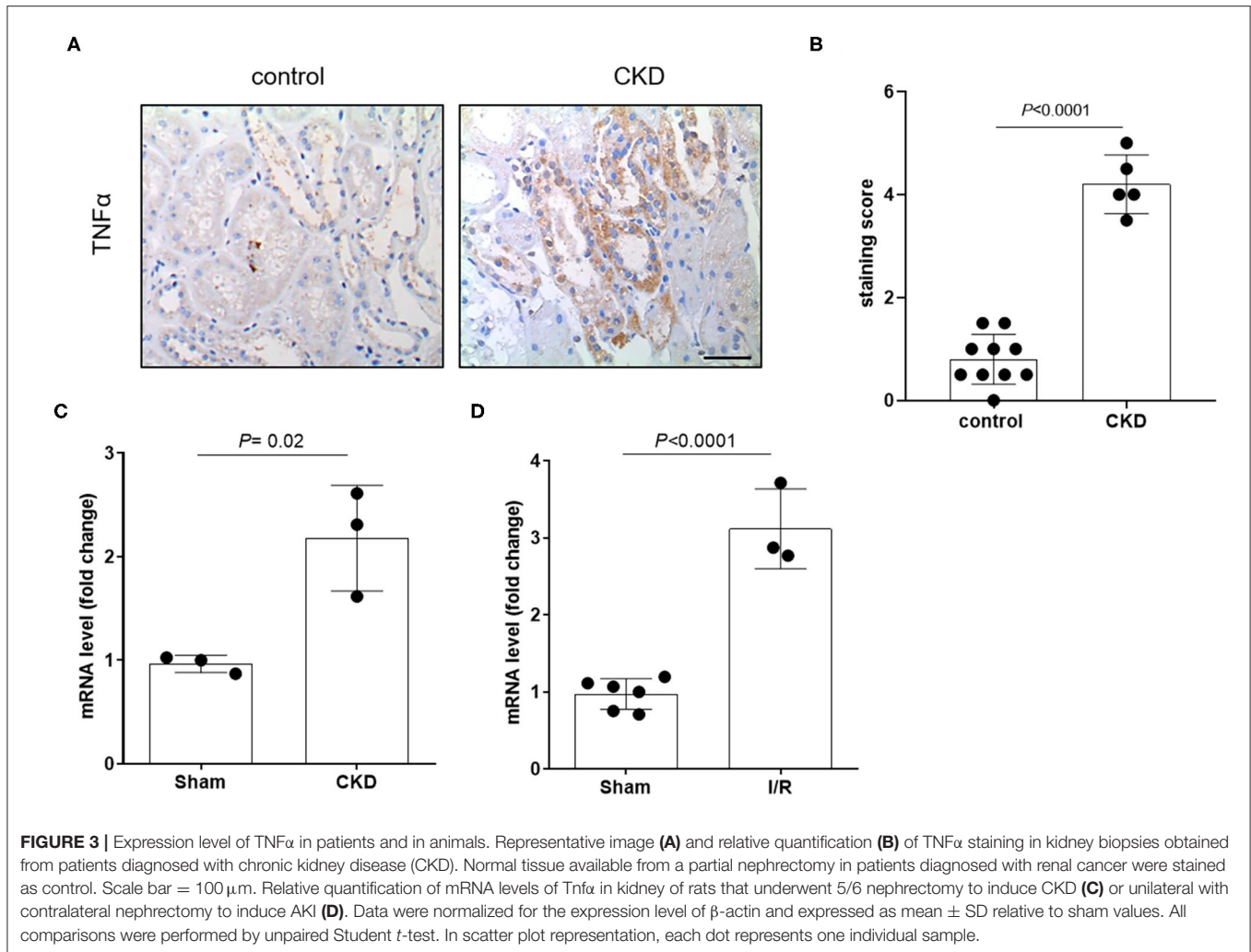
The part of the sequence of the human OCT2 promoter containing the predicted NF- κ B binding site was cloned from a commercially available human genomic library (G1471, Promega, Fitchburg, WI, USA) using the forward primer 5'-ccgacggctctgttgggttg-3' and the reverse primer 5'-gcgcaaggtagccgagagcaga-3'. A 689-bp amplicon corresponding to the -586 / +103 fragment flanking the transcription starting site of the *SLC22A2* gene was isolated and blunt ligated into the pTarget vector (A1410, Promega, Madison, WI, USA). The insert was then subcloned in pMCS-Luc vector (16146, ThermoFisher scientific, Waltham, MA, USA) by digestion with *NheI* and *SmaI* restriction enzymes. Mutants were generated using *AvaII* restriction enzyme to generate the Δ 327 construct or the *FauI* restriction enzyme to generate the Δ 363 vector. The TTTCAAAA-mutant was generated by QuikChange Site-Directed Mutagenesis Kit (A13282, ThermoFisher, La Jolla, CA, USA).

Luciferase Assay

RPTEC cells were seeded into 24-well plates at a density of 1.5×10^5 cells/well. Cells were transfected using Lipofectamine 2000 (ThermoFisher scientific, Waltham, Massachusetts, USA), with 600 ng of DNA and 30 ng of a control vector for transfection efficiency, using the constitutive expression pCMV-Red Firefly Luc Vector (16156, ThermoFisher scientific, Waltham, MA, USA). After transfection, cells were exposed to 10 ng TNF α (<0.1 endotoxin units per μ g of protein, 210-TA, R&D Systems, Minneapolis, MN, USA) or the vehicle control for 24h and then luciferase activity was determined using a Gaussia-firefly luciferase dual assay kit (16181, ThermoFisher scientific, Waltham, MA, USA) and a LB940 luminometer (Berthold, Germany).

NF- κ B p65 Transcription Activity Assay

NF- κ B p65 DNA binding activity from nuclear extracts were determined by using ELISA-based colorimetric kit from Abcam (ab133112, Cambridge, UK) as previously described (31).



Nuclear extracts from cells were incubated overnight with a double stranded DNA sequence containing the NF- κ B response element prior ELISA.

Statistics

Data are expressed as mean \pm S.D. Statistical significance of differences between groups was assessed by Student's *t*-test or one-way ANOVA test. GraphPad (RRID: SCR_002798) was used to perform statistical analyses.

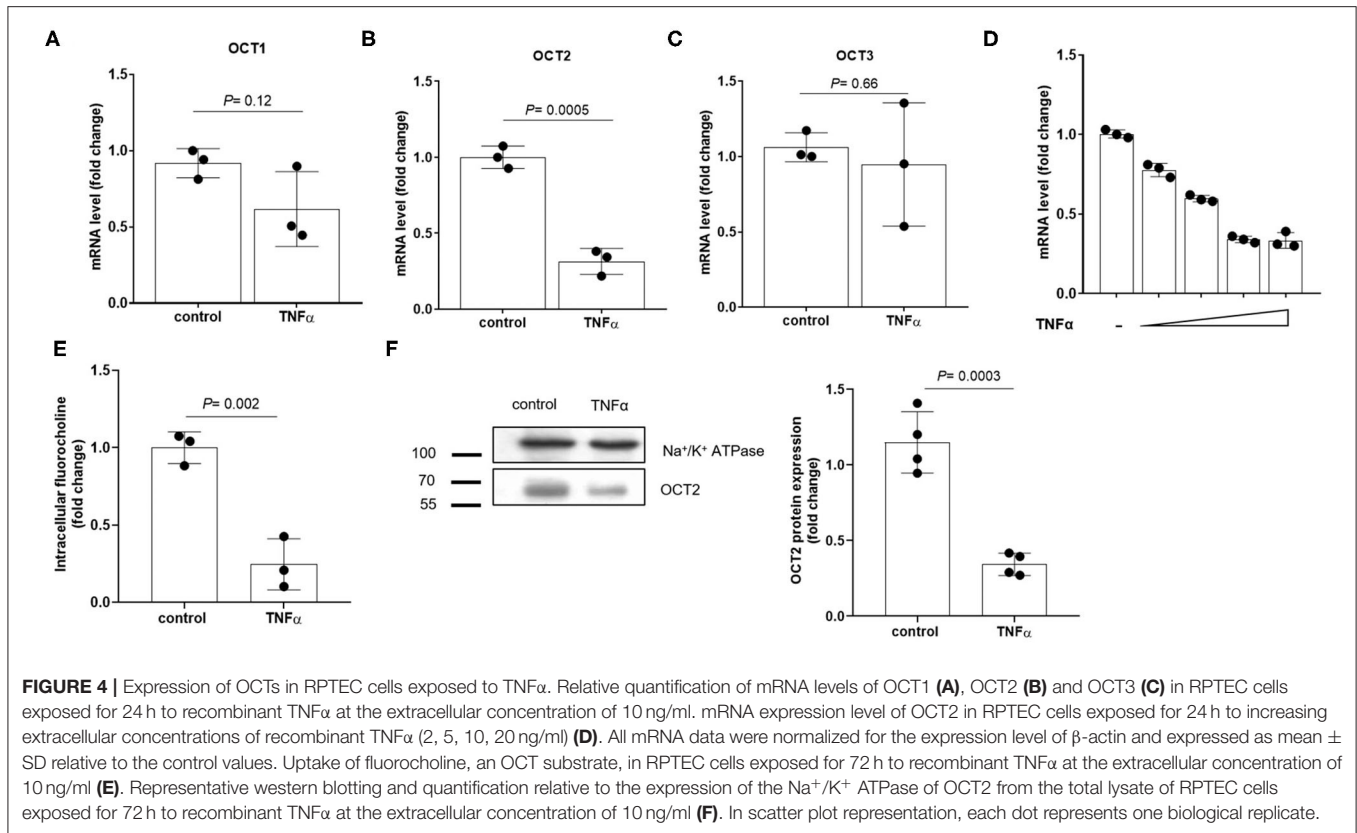
Study Approval

Animal protocols, and experiments abided by the laws of animal protection and were approved by the local institutional animal committee of Xuzhou Medical University, China (license number 20210102W001). The handling of human samples followed the Declaration of Helsinki guidelines regarding ethical principles for medical research involving human subjects. Written informed consent was provided by each patient participated in the research. The study protocol was approved by the Scientific Ethical Committee of Liaocheng University/Liaocheng People's Hospital, China (license number 2020022).

RESULTS

Organic Cation Transporter Expression Level in CKD and AKI

The protein expression level of OCT2 and OCT3, the two main polyspecific organic cation transporters expressed in human proximal tubular cells, was evaluated in biopsies from patients diagnosed with CKD and compared to that in pathologically normal renal tissue collected from patients with normal kidney function who underwent surgery for suspicion of renal cancer. **Figure 1** shows that OCT2 and OCT3 localized on the basolateral membrane of proximal tubular cells and that the protein expression level of both transporters was markedly lower in the kidney of CKD patients than that in the kidney of patients with normal renal function. The subcortical origin of the bioptic tissues was determined by assessing the mRNA level of the sodium/glucose cotransporter 1 (SGLT1), whose expression is confined to segment 3 of the cortex, and the sodium/glucose cotransporter 2 (SGLT2), expressed in segments 1 and 2, where OCT2 and OCT3 primarily localize. In **Figure 1B**, it can be seen that the mRNA level of SGLT2 exceeded that of SGLT1



by approximately 50-fold in the control as well as in the CKD group, supporting the outer cortex origin of the samples. Analogously, in line with previous reports (3, 15–17), Oct2 mRNA expression level was markedly reduced in CKD and I/R-induced rats (Figure 2). Conversely, the renal expression level of TNF α , localized in the cytoplasm, was significantly increased in patients diagnosed with CKD (Figures 3A,B) as well as in rats with CKD or AKI (Figures 3C,D).

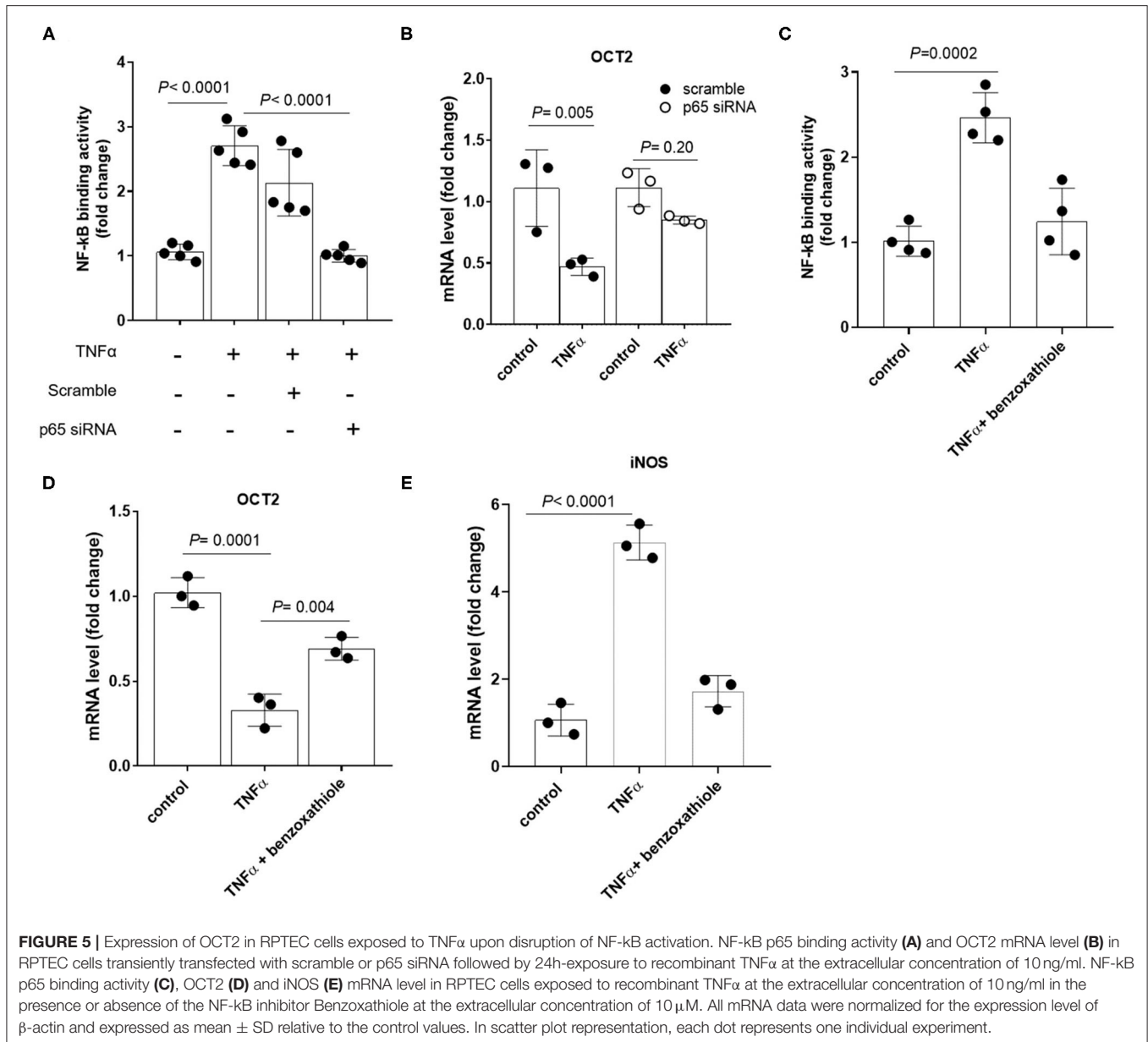
Effect of Recombinant TNF α on the OCT Expression in RPTEC Cells

To investigate the existence of a causal link between TNF α induction and the repression of OCTs, RPTEC cells were exposed to recombinant TNF α at the extracellular concentration of 10 ng/ml. In Figure 4 it can be seen that OCT2 mRNA level (B) was markedly decreased in the presence of TNF α , whereas those of OCT1 (A) and OCT3 (C) did not significantly change. The TNF α -induced downregulation of OCT2 was dose-dependent with maximal response at 10 ng/ml (Figure 4D). To investigate the impact of TNF α on OCT2 function, the protein expression level and the transport of fluorocholine, primarily an OCT2 substrate (32, 33), was assessed upon 72 h-exposure to recombinant TNF α at the extracellular concentration of 10 ng/ml. It can be seen that fluorocholine uptake (Figure 4E) as well as OCT2 protein level (Figure 4F) were markedly reduced by the incubation with TNF α . Thus far, the inhibitory effect of pro-inflammatory cytokines on OCT2 expression was

considered a post-translational modulation, however, the present data suggest that TNF α also repressed the transcription of the *SLC22A2* gene, encoding for OCT2 (34). Being NF- κ B the primary genuine transcription factor activated by TNF α , its role in TNF α -induced OCT2 downregulation was investigated. The incubation with recombinant TNF α enhanced NF- κ B binding activity in RPTEC cells (Fig. 5A). To assess whether the TNF α -induced OCT2 downregulation was NF- κ B dependent, the NF- κ B transcriptional activity was disrupted by silencing p65, the NF- κ B subunit responsible for initiating transcription subunit (Figure 5B). The repressing effect of recombinant TNF α on OCT2 gene expression was abolished in RPTEC cells transiently transfected with a siRNA against p65, indicating that the downregulation of OCT2 expression upon exposure to TNF α required a functional NF- κ B. Similarly, treatment of RPTEC cells with benzoxathiole, a NF- κ B inhibitor (Figure 5C), partially restored the OCT2 gene expression in the presence of recombinant TNF α (Figure 5D). Notably, benzoxathiole also almost fully abolished the induction of the expression of the inducible nitric oxide synthase (iNOS), which was previously shown to reduce OCT2 protein expression (Figure 5E) (34).

Identification of NF- κ B Binding Site in OCT2 Promoter

In rat Oct2 promoter region, a putative NF- κ B binding site in position –143 to –129 was previously predicted (26). Based on sequence alignments, a similar sequence was found in

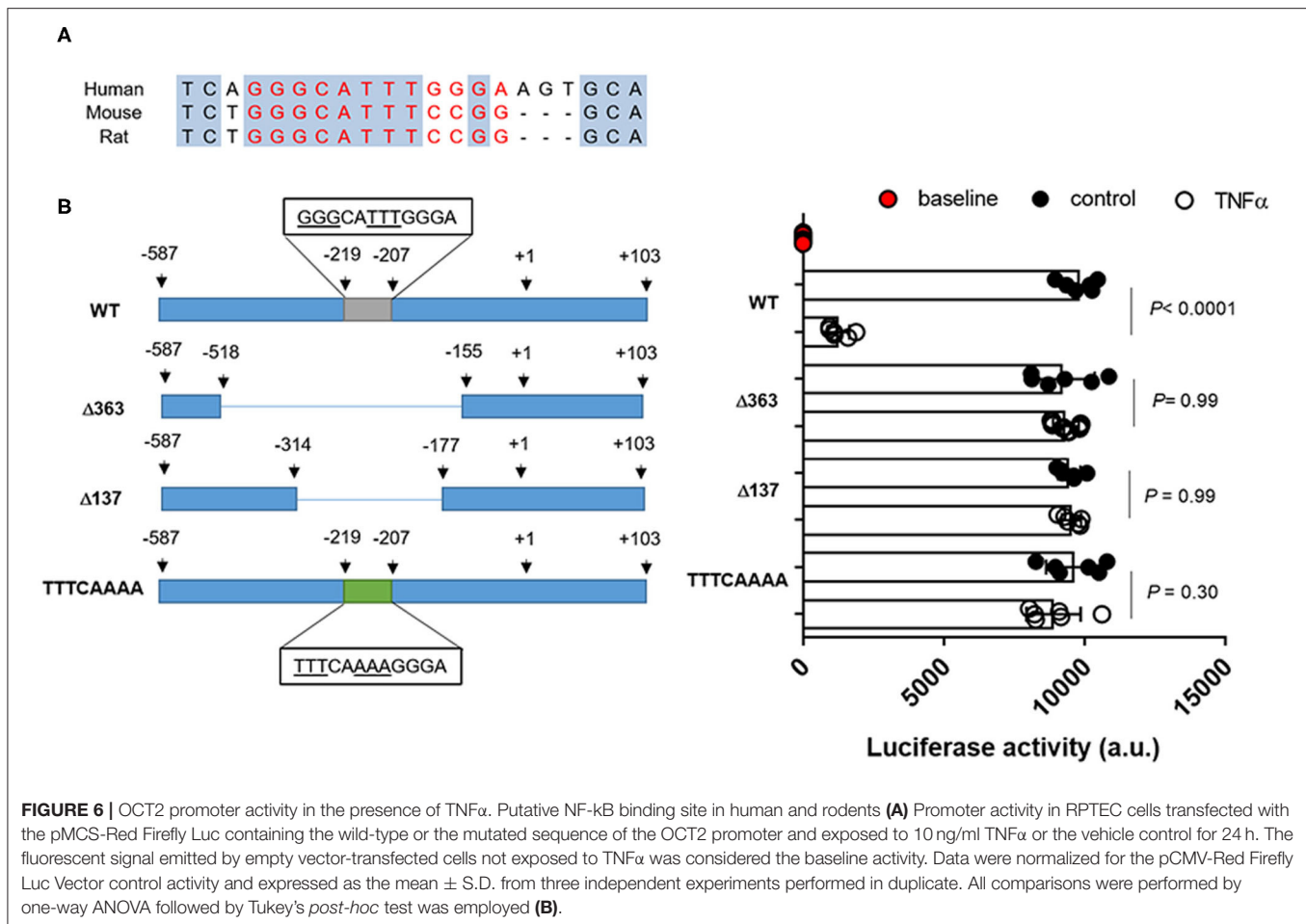


position–219 to–207 of the promoter region of the human OCT2 (**Figure 6A**). As shown in **Figure 6B**, the activity of the OCT2 promoter region containing the putative NF- κ B binding site was significantly suppressed in the presence of recombinant TNF α . Such inhibition was abolished by deleting or mutating the putative NF- κ B binding site (**Figure 6B**).

Effect of LPS Injection on Organic Cation Secretion

To assess the direct effect of inflammation on OCT expression and organic cation tubular secretion, rats were treated with LPS to induce acute inflammation. In **Figure 7** it can be seen that the mRNA (**Figure 7B**) and the protein (**Figure 7E**) of Oct2 was significantly reduced by the treatment with LPS, conversely,

the mRNA (**Figure 7A**) and protein (**Figure 7E**) level of Tnf α was markedly higher in the kidney of rats exposed to LPS. The kidney mRNA level of Mate1 and Mdr1 (P-glycoprotein), two apical transporters often coupled to Oct2 in the vectorial transport of cations, was not significantly different between LPS and control animals (**Figures 7C,D**). To investigate whether the LPS injection affected the renal handling of organic cations, the fluorescent cationic compound Rhodamine 123 was used as a tracer. Rhodamine 123 has been previously shown to be substrate of OCT2 (35), which mediates Rhodamine 123 tubular secretion (34, 36). The renal handling of Rhodamine 123 was determined in the kidney 12 h after the last injection of LPS. **Figure 7F** shows that the accumulation of Rhodamine 123 over time exceeded that of tetramethyl Rhodamine isothiocyanate (TRITC)-conjugated



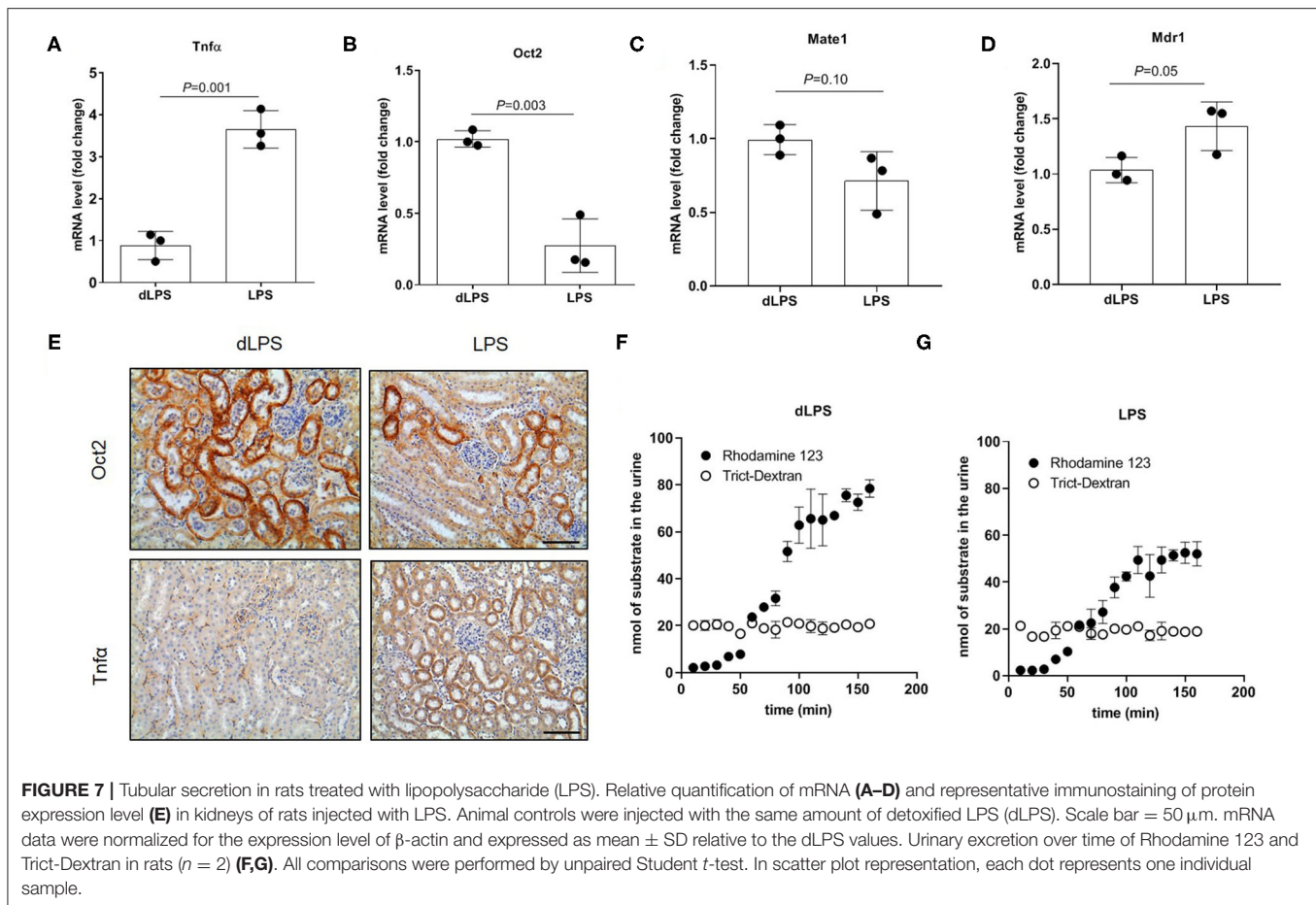
dextran (TRITC-Dextran), a fluorescent high molecular mass dextran which is not reabsorbed and is considered a practical read-out of glomerular filtration (37). It can be seen that, at steady-state level, the renal clearance of Rhodamine 123 was close to 4 times that of TRITC-Dextran, indicating that Rhodamine 123 is actively secreted by the tubule. The steady-state renal clearance of Rhodamine 123 in the rats treated with LPS was roughly 60% of that in rats treated with dLPS (Figures 7F,G).

DISCUSSION

The expression of oct2 and cation active tubular secretion is often altered in various animal models of kidney disease. In rats, oct2 expression level and the clearance of the quaternary ammonium cation Tetraethylammonium (TEA), a prototypical OCT substrate, decreased after induction of acute kidney injury by ischemia-reperfusion procedure (17). Similarly, 5/6 nephrectomized rats developing chronic renal failure had lower expression of oct2 and slower tubular secretion of cimetidine, another OCT substrate (3). Oxonate-induced hyperuricemic rats (15), as well as diabetic rats (16) displayed a downregulation of oct2. Conversely, our groups showed that obese individuals

and mice fed a high-fat diet with chronic kidney disease had a higher expression of renal oct2 than lean ones (18). The present work demonstrates that acute inflammation in the frame of normal renal function is sufficient to repress the expression of oct2, resulting in a slower active tubular secretion of the fluorescent cation Rhodamine 123. OCT2 mediates the transport of the substrate from the peritubular blood into the proximal tubular cells, and works in tandem with the multidrug extrusion transporter 1 (MATE1) and with efflux pump such as P-gp, both located on the brush-border membrane of the proximal tubular cells (38, 39). While MATE1 has been previously shown to be downregulated by TNF α (40), no significant change in MATE1 mRNA level was observed when RPTEC cells were exposed to TNF α (not shown), nor in the kidney of rats treated with LPS (Figure 7C). Similarly, we did not observe LPS-mediated induction of the mRNA level of P-gp as previously reported (30). Taken together, our data indicate that OCT2 is rate limiting in Rhodamine 123 vectorial transport across the proximal tubule (12, 41–43).

The effect of endotoxemia on Oct-mediated tubular secretion has been previously put forth by Heemskerk and colleagues, who demonstrated that aminoguanidine, an inhibitor of the inducible nitric oxide synthase (iNOS), rescued the tubular



secretion of Rhodamine 123 and the protein expression of oct1 and oct2, but not that of their mRNA, in the kidney of Wistar Hannover rats injected with LPS (34). The induction of iNOS expression upon binding and nuclear translocation of NF- κ B (**Figure 5E**) (44), boosts the conversion of L-arginine into nitric oxide, a gas that mediates multiple biochemical processes, including the activation of the guanylate cyclase, thereby the increase synthesis of cyclic guanosine monophosphate (cGMP) a signaling molecule that was previously shown to be involved in the inhibition of the activity of rat oct1 and human OCT2 *in vitro* (45–47). Here, we show that the effect of TNF α on OCT2 expression was abolished upon silencing of p65, the NF- κ B subunit responsible for initiating transcription and upon pharmacological inhibition of NF- κ B with benzoxathiole. This suggests that TNF α -induced downregulation of OCT2 occurs exclusively via NF- κ B and it is the result of the iNOS-independent *SLC22A2* gene transcription repression and the iNOS-dependent reduced protein stability, with only the latter rescued by the treatment with aminoguanidine (34).

In the present work, we focused on elucidating the renal TNF α -OCTs axis, yet the repressive effect of pro-inflammatory cytokines has been reported for various other transporters including bile acid transporters, organic anion transporters

(OATs), organic anion transporting polypeptides (OATPs) and OCT1 (48). Therefore, the reduction in tubular secretion induced by inflammation may not be restricted to organic cation, but rather encompasses a wider spectrum of solutes, including various putative uremic toxins observed in patients with CKD (49). Genetic, life-style and environmental factors can directly trigger the disease, which slowly creates a highly oxidative uremic milieu that fosters the inflammatory cycle and worsens the residual renal function (50). Notably, inhibitors of the renin–angiotensin–aldosterone system, such as angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs), which are widely prescribed in the management of CKD, seems also to reduce the production of pro-inflammatory products by, at least in part, inhibiting the NF- κ B pathway (24). In a similar vein, the inhibition of prostaglandin synthesis by non-steroidal anti-inflammatory drugs (NSAIDs) increased the tubular solute reabsorption in healthy volunteers (51). In line with this, administration of IL-1 receptor antagonist in hemodialysis patients significantly reduced systemic inflammation markers and the urinary loss of albumin (52). In line with these clinical observations, our previous study demonstrated that genetically TNF α -deficient mice fed a high-fat diet were resistant

to renal inflammation and oxidative stress in comparison with animals fed a normal diet (53). An efficient control of the local inflammatory status may sustain the tubular secretion of protein bound uremic toxins escaping the hemodialysis, which, despite major technological advances, remains a major limitation of today's renal replacement therapy (49).

NEW AND NOTEWORTHY

Despite the advance of the technology, hemodialysis and hemofiltration are not able to compensate for the reduced active tubular secretion of protein-bound substances suffered by patients with kidney dysfunction. Our experimental results coupled to the observed association between OCT expression and pro-inflammatory cytokines in the kidneys of patients diagnosed with chronic kidney disease; provide the rationale for implementing a long-term anti-inflammatory therapy alongside the standard renal replacement therapy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Scientific Ethical Committee of Liaocheng University/Liaocheng People's Hospital, China (license number 2020022). The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by the Local Institutional Animal Committee of Xuzhou Medical University, China (license number 20210102W001).

AUTHOR CONTRIBUTIONS

CH, JZ, and LC: conception and design. CH, JZ, FW, QL, QC, AH, MV, and ZG: methodology and data acquisition. CH, JZ, GK-U, and LC: data interpretation. CH and LC: writing of the initial draft. JZ, FW, QL, QC, AH, MV, GK-U, and ZG: revision and editing. All authors have read and approved the final manuscript.

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