Received: 26 April 2022 | Accepted: 9 May 2022

DOI: 10.1002/prp2.973

ORIGINAL ARTICLE

EOS789, pan-phosphate transporter inhibitor, ameliorates the progression of kidney injury in anti-GBM-induced glomerulonephritis rats

Yoshinori Tsuboi[1] | Yasuhiro Ichida[1] | Atsuko Murai[2] | Akira Maeda[1] | Manami Iida[1] | Atsuhiko Kato[2] | Shuichi Ohtomo[2] | Naoshi Horiba[1]

[1]Research Division, Chugai Pharmaceutical Co., Ltd., Gotemba, Shizuoka, Japan

[2]Translational Research Division, Chugai Pharmaceutical Co., Ltd., Gotemba, Shizuoka, Japan

Correspondence
Yoshinori Tsuboi, Komakado 1-135, Gotemba, Shizuoka 412-8513, Japan.
Email: tsuboiysn@chugai-pharm.co.jp

Abstract

Hyperphosphatemia associated with chronic kidney disease (CKD) not only dysregulates mineral metabolism and bone diseases, but also strongly contributes to the progression of kidney disease itself. We have identified a novel drug for hyperphosphatemia, EOS789, that interacts with several sodium-dependent phosphate transporters (NaPi-IIb, PiT-1, and PiT-2) known to contribute to intestinal phosphate absorption. In this study, we investigated whether EOS789 could ameliorate kidney disease progression in glomerulonephritis rats. Anti-glomerular basement membrane (GBM) nephritis was induced in rats by intravenously administering two types of anti-rat GBM antibodies. We evaluated the effect of EOS789 administered in food admixture on hyperphosphatemia and kidney disease progression. In an anti-GBM nephritis rats, which exhibit a significant increase in serum phosphate and a decline in renal function, EOS789 dose-dependently improved hyperphosphatemia and EOS789 at 0.3% food admixture significantly ameliorated kidney dysfunction as shown in the decline of serum creatinine and BUN. Renal histopathology analysis showed that EOS789 significantly decreased crescent formation in glomeruli. To elucidate the mechanism underlying glomerular disease progression, human mesangial cells were used. High phosphate concentration in media significantly increased the expression of Collagen 1A1, 3A1, and αSMA mRNA in human mesangial cells and EOS789 dose-dependently suppressed these fibrotic markers. These results indicate that EOS789 prevented glomerular crescent formation caused by mesangial fibrosis by ameliorating hyperphosphatemia. In conclusion, EOS789 would not only be useful against hyperphosphatemia but may also have the potential to relieve mesangial proliferative glomerulonephritis with crescent formation.

Abbreviations: CKD, chronic kidney disease; Col1A1, collagen type 1 α1; Col3A1, collagen type 3 α1; G3P, Glycerol-3-phosphate; GBM, glomerular basement membrane; NaPi-IIb, sodium-dependent phosphate transporter, Slc34a2; PiT-1, sodium-dependent phosphate transporter, Slc20a1; PiT-2, sodium-dependent phosphate transporter, Slc20a2; PTH, parathyroid hormone; αSMA, α-smooth muscle actin.

Primary laboratory of origin: Chugai Pharmaceutical Co., Ltd.

Recommended section: Drug Discovery and Translational Medicine

KEYWORDS
anti-GBM nephritis rats, crescent, EOS789, hyperphosphatemia

1 | INTRODUCTION

Hyperphosphatemia is commonly induced by impaired urinary phosphate excretion in chronic kidney disease (CKD).[1] It causes aberrant bone metabolism and has been consistently associated with increased morbidity and mortality.[2–5] Although blood phosphate is mainly regulated by renal excretion in healthy kidneys, intestinal phosphate absorption largely contributes to this regulation in kidney failure. Phosphate binders have been used to ameliorate cardiovascular calcification for hyperphosphatemia, secondary hyperparathyroidism, and other conditions.[6–10] In several rodent CKD models, phosphate binders or phosphate restriction in food are reported to improve not only hyperphosphatemia but also the progression of kidney disease itself.[11–15] A high concentration of phosphate is reported to increase intra-glomerular pressure and induce endothelial mesenchymal transition or extracellular matrix accumulation in mesangial cells.[16,17] This indicates that hyperphosphatemia is one of the main contributors to kidney disease progression and that the amelioration of hyperphosphatemia could suppress.

EOS789, a pan-phosphate transport inhibitor, is now being developed for hyperphosphatemia associated with kidney failure. EOS789 inhibits intestinal phosphate transporters, i.e. Slc34a2 (NaPi-IIb), 20a1 (PiT-1), and 20a2 (PiT-2), and ameliorates hyperphosphatemia in humans and rats with chronic kidney disease (CKD).[18,19] EOS789 not only reduced serum phosphate concentration but also ameliorated disease progression in a rodent CKD model induced by the injection of anti-Thy1.1 antibody, followed by unilateral nephrectomy.[18] This model mainly exhibits severe tubulo-interstitial fibrosis. Thus, although EOS789 ameliorates both tubular-interstitial fibrosis and glomerular sclerosis in this model, the precise effects of EOS789 on glomerular disease itself remains unknown. Anti-glomerular basement membrane antibody-induced nephritis rats (anti-GBM rats) have been widely used as a glomerulonephritis model. It primarily exhibits severe glomerular sclerosis and crescent formation.[20] We established this model by injecting two types of anti-GBM antibody, that induced histopathological changes similar to those reported previously.

In this study, we evaluated the effect of EOS789 on glomerular disease using anti-GBM rats and in vitro mesangial cells.

Significance Statement

EOS789, which is currently being evaluated in clinical studies for the treatment of hyperphosphatemia (ClinicalTrials.gov number NCT02965053, JAPIC ID JapicCTI-152992), could ameliorate not only hyperphosphatemia but also kidney disease progression in anti-GBM nephritis rats. EOS789 inhibits phosphate uptake in mesangial cells, which prevents the crescent formation in glomerulonephritis. EOS789 could provide a significant benefit to patients with mesangial proliferation glomerulonephritis.

2 | MATERIALS AND METHOD

2.1 | Chemicals

2.1.1 | EOS789

[7-[[2,3-difluoro-4-[2-[2-methoxyethyl(methyl)amino]ethoxy]phenyl]methyl]-10-hydroxy-6-methyl-8-oxo-N-[4-(trifluoromethyl)-2-[6-(trifluoromethyl)pyrimidin-4-yl]phenyl]-6,7-diazaspiro[4.5]dec-9-ene-9-carboxamide;4-methylbenzenesulfonic acid; Figure 1] was synthesized by Chugai Pharmaceutical Co., Ltd. Details of the synthesis are described in WO2014142273 (Example 14).[21]

2.2 | Animal experiments

Male Wistar rats were purchased from the Jackson Laboratories Japan, Inc. (Tokyo, Japan). Animal procedures and protocols were in accordance with the Guidelines for the Care and Use of Laboratory Animals at Chugai Pharmaceutical Co. Ltd. and approved by the Institutional Animal Care and Use Committee (Approved No. 20–155). The anti-GBM rats were established by intravenously administrating two types of anti-GBM antibodies (Nephrotogenic Monoclonal Antibody a84 and b35, Chondrex Inc., Woodinville, WA) at 300 μg/head to 7-week old Wistar rats. Immediately before disease induction rats were allocated to normal control (n = 6), disease control, or EOS789 treatment groups

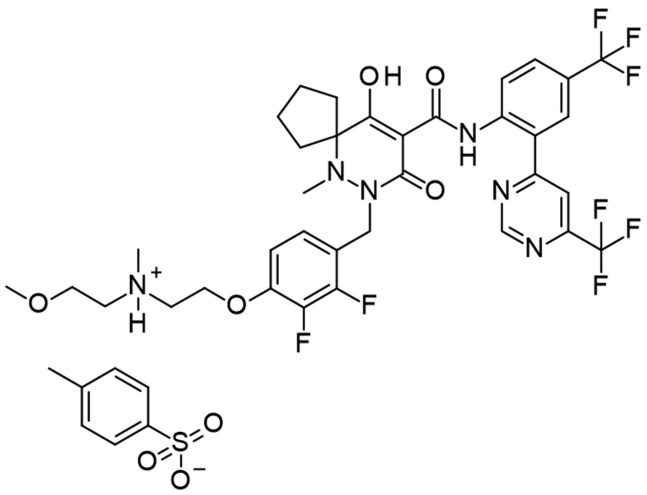

FIGURE 1 Chemical structure of EOS789 (tosylate form)

(n = 11, respectively) based on body weight, serum phosphate, creatinine, or urinary phosphate excretion. Rats were fed on powdered food (CE-2; Ca 1.18%, P 1.10%; CLEA Japan, Inc., Shizuoka, Japan) containing EOS789 0%, 0.15%, or 0.3% ad libitum. Animals were treated with EOS789 immediately after disease induction to show the preventive effects of EOS789 in kidney failure. The daily doses of EOS789 were around 100 and 200 mg/kg, respectively, which were much less than phosphate binders used in preclinical studies.[22,23] Five weeks after the treatment they were subjected to analysis. Blood, urine, feces samples, and tissue were collected 5 weeks after disease induction. Blood, urine, feces, and tissue samples were collected as described below.

Blood samples were collected from the abdominal portion of the vena cava under isoflurane anesthesia, transferred into the MiniCollect tubes and centrifuged (1630 g, 15 min, 4°C) to prepare serum samples. Urine samples were diluted with an equal amount of 1 mol/L HCl and centrifuged to collect the supernatant (1630 g, 15 min, 4°C). Urinary excretion of Pi, albumin, and sodium were calculated as fractional excretion to minimize the effect of GFR increase. Feces samples were suspended in 0.5 mol/L hydrochloric acid (HCl) and centrifuged to collect the supernatant (1630 g, 15 min, 4°C). The animals were euthanized by exsanguination under isoflurane and kidney tissue were collected. The collected kidney tissues were dissected for pathology (formalin-fixed and methylcarnoy-fixed) and mRNA expression analysis (for mRNA, only the cortex region was collected and frozen by liquid nitrogen).

2.3 | Biochemical analyses

Phosphate, calcium, creatinine, urea nitrogen (BUN), albumin, and electrolytes in serum, urine, or feces were measured by an automatic analyzer (TBA-2000FR, TOSHIBA Co., Tokyo, Japan). Serum calcitriol (1,25(OH)2D3 RIA Kit FR, Fujirebio, Inc., Tokyo, Japan), intact parathyroid hormone (PTH) (Immutopics Rat Intact PTH ELISA kit, Quidel Corporation, San Diego, CA), FGF23 (FGF-23 ELISA Kit, KAINOS Laboratories, Inc., Tokyo, Japan), and Glycerol-3-phosphate (G3P) (Amplite™ Colorimetric G3P Assay Kit, AAT Bioquest, Inc., Sunnyvale, CA) were measured according to the manufacturer's instructions.

2.4 | Pathological analysis

Kidney tissues were fixed with 10% neutral buffered formalin solution (for hamatoxyline-eosin (HE) staining) or Methyl Carnoy's fixative (for periodic acid-Schiff (PAS) staining) and then embedded in paraffin. The paraffin blocks were cut into 4 μm thick sections and stained for HE or PAS. Crescent formation was evaluated by counting glomeruli in half the total area of kidney from each rat on PAS-stained sections. Interstitial fibrosis was graded for each rat on HE-stained sections; score 0 represents no lesion, score 1 represents lesions involving the focal area.

2.5 | Gene expression analysis

Kidney total RNA samples were extracted from frozen kidney cortex samples using an RNaesy Mini kit (QIAGEN). cDNA samples were synthesized from RNA samples using a reverse transcription kit (Roche Applied Science). Gene expression levels were measured by real-time PCR, using TaqMan probe/primers sets (Applied Biosystems) for each gene.

2.6 | Cells

Primary human mesangial cells were purchased from Cambrex Co. (Walkersville, MD). Cells were first immortalized by the transduction of lentivirus encoding simian virus 40 T antigen, which possessed the temperature sensitive tsA58 mutation.[24] The immunized clone was re-infected with lentivirus encoding human telomerase reverse transcriptase. One clone #130hT-9 (hMes 130hT-9) which was characterized as having the profile of a mesangial cell, for example, induction of α-smooth muscle actin (αSMA), collagen 1A1 (Col1A1), and serpine (PAI-1) by TGFβ, was selected and used for this study.[25]

Reverse-transcribed PCR was conducted to reveal the expression of NaPi-IIa, -b, -c, PiT-1, and PiT-2 mRNA in hMes 130hT-9. cDNA samples were synthesized as described in the above section. These single-stranded DNA fragments were amplified with primer sets specific for each gene, as shown in Table S1.

Procedures using human mesangial cells were in accordance with the Declaration of Helsinki guidelines and approved by the Institutional Review Board of Chugai Pharmaceutical Co. Ltd. (approval number: 682).

2.7 | In vitro experimental design

hMes 130hT-9 cells were seeded onto a 24-well plate and were cultured overnight. After reaching subconfluence, cells were cultured in serum-restricted medium (0.5% FBS) containing high phosphate (5 mM) with or without EOS789 for 24 h. Total RNA was extracted from the cell lysate and each gene was measured as described in the above section.

2.8 | Statistical analysis

Statistical analysis was performed using JMP 15.0.0 (SAS Institute Inc., Cary, NC). For the in vivo and in vitro studies, the Welch test or parametric Williams test were conducted.

2.9 | Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.

org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[26] and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

3 | RESULTS

3.1 | Effect of EOS789 on anti-GBM rats

3.1.1 | Phosphate metabolism

Serum Pi was significantly increased in disease rats and EOS789 treatment significantly and dose-dependently suppressed this increase (Figure 2A). Parallel to serum, EOS789 at 0.3% food admixture significantly increased fecal Pi excretion (68.4 ± 7.8 mg/day for disease control and 89.1 ± 4.5 mg/day for EOS789 at 0.3% food admixture, $p < .05$) and decreased fractional urinary Pi excretion compared with disease control (41.7 ± 5.0% for disease control and 18.5 ± 1.9% for EOS789 at 0.3% food admixture, $p < .05$) (Figure 2B,C). There was no change in serum calcium (data not shown). In addition, increase in serum FGF23 in diseased rats was significantly suppressed by EOS789 (Figure 2D). On the other hand, PTH was apparently increased by disease induction but not suppressed by EOS789 (Figure 2E). Serum calcitriol was severely suppressed in the disease condition but was significantly improved by EOS789 (Figure 2F). These results indicate that EOS789 inhibited intestinal Pi absorption via NaPi-IIb, PiT-1 and/or PiT-2, leading to the amelioration of hyperphosphatemia and its related phosphate metabolism in anti-GBM rats.

3.2 | Kidney function and histopathology

Anti-GBM rats showed a significant increase of serum creatinine, BUN, and urinary fractional excretion of albumin. EOS789 at 0.3% food admixture significantly decreased serum creatinine (1.97 ± 0.32 mg/dL for disease control and 1.31 ± 0.17 mg/dL for EOS789 at 0.3% food admixture, $p < .05$) and appeared to decrease BUN (124 ± 26 mg/dL for disease control and 77 ± 9 mg/dL for EOS789 at 0.3% food admixture) at 5 weeks after disease induction (Figure 3A,B), although this was non-significant. Urinary fractional excretion of albumin was also significantly reduced by EOS789 treatment (0.07 ± 0.01% for disease control and 0.04 ± 0.01% for EOS789 at 0.3% food admixture, $p < .05$) (Figure 3C). In addition, EOS789 at 0.3% food admixture significantly decreased serum sodium (141.7 ± 0.4 mEq/L for disease control and 140.5 ± 0.4 mEq/L for EOS789 at 0.3% food admixture, $p < .05$) and urinary fractional excretion of sodium (1.84 ± 0.21% disease control and 1.15 ± 0.19% for EOS789 at 0.3% food admixture, $p < .05$) (Figure 3D).

Figure 4 shows the histopathology of kidney. Anti-GBM rats showed a significant increase of crescent formation and mild tubule-interstitial fibrosis. Although EOS789 did not improve the tubule-interstitial fibrosis (Figure 4E-H), it significantly decreased the glomerular crescent formation (78.3 ± 4.4% of crescent in total glomeruli for disease control and 66.1 ± 3.3% for EOS789 at 0.3% food admixture, $p < .05$) (Figures 4A-D, 5). There was a strong positive correlation between crescent formation and serum creatinine in the anti-GBM rats (Figure 6), which indicates that EOS789 ameliorated the glomerular crescent formation, leading to the improvement of kidney function.

In addition, body weight was significantly increased in the EOS789 treatment group, which confirmed the improvement of the general condition with regards to uremia (Figure S1).

3.3 | FGF23 related factors in serum or kidney

G3P is reported to stimulate FGF23 production in bone.[27] Serum G3P was significantly increased by disease induction and this increase tended to be suppressed by EOS789 (Figure S2).

FGF23 is reported to be synthesized not only in bone but also in kidney.[28] Its synthesis is induced by tumor necrosis factor (TNF). Renal mRNA of FGF23, TNFα, and Nurr1 which is down stream of TNF[29,30] were significantly increased in diseased rats. EOS789 could not suppress the induction of these molecules (Table S2).

These results indicate that the increase of serum FGF23 was caused not only by hyperphosphatemia but also by renal G3P synthesis or renal inflammation in the anti-GBM rats, and that EOS789 would also inhibit G3P-mediated FGF23 production.

3.4 | In vitro analysis using human mesangial cells

hMES 130hT expressed PiT-1 and PiT-2 mRNA but not Slc34a (NaPi-IIa, b, or c) mRNA (Figure 7). The high phosphate condition increased fibrotic markers, that is, mRNA expression of collagen 1A1, 3A1, or αSMA, which were dose-dependently inhibited by EOS789 (Figure 8).

4 | DISCUSSION

In the present study, we demonstrated that EOS789 ameliorated not only hyperphosphatemia but also kidney injury in anti-GBM rats. EOS789 significantly increased fecal Pi excretion, which leads to the decrease of serum Pi concentration and urinary Pi excretion. These results indicate that EOS789 inhibited intestinal Pi absorption by inhibiting intestinal phosphate transporters, that is, NaPi-IIb, PiT-1, and/or PiT-2, in anti-GBM rats. In addition, EOS789 significantly reduced the serum creatinine and urinary fractional excretion of albumin compared with the diseased control rats. Histopathological analysis showed that EOS789 significantly reduced crescent formation without ameliorating tubule-interstitial injury. These results indicate that EOS789 improved kidney function by directly ameliorating glomerular disease in anti-GBM rats.

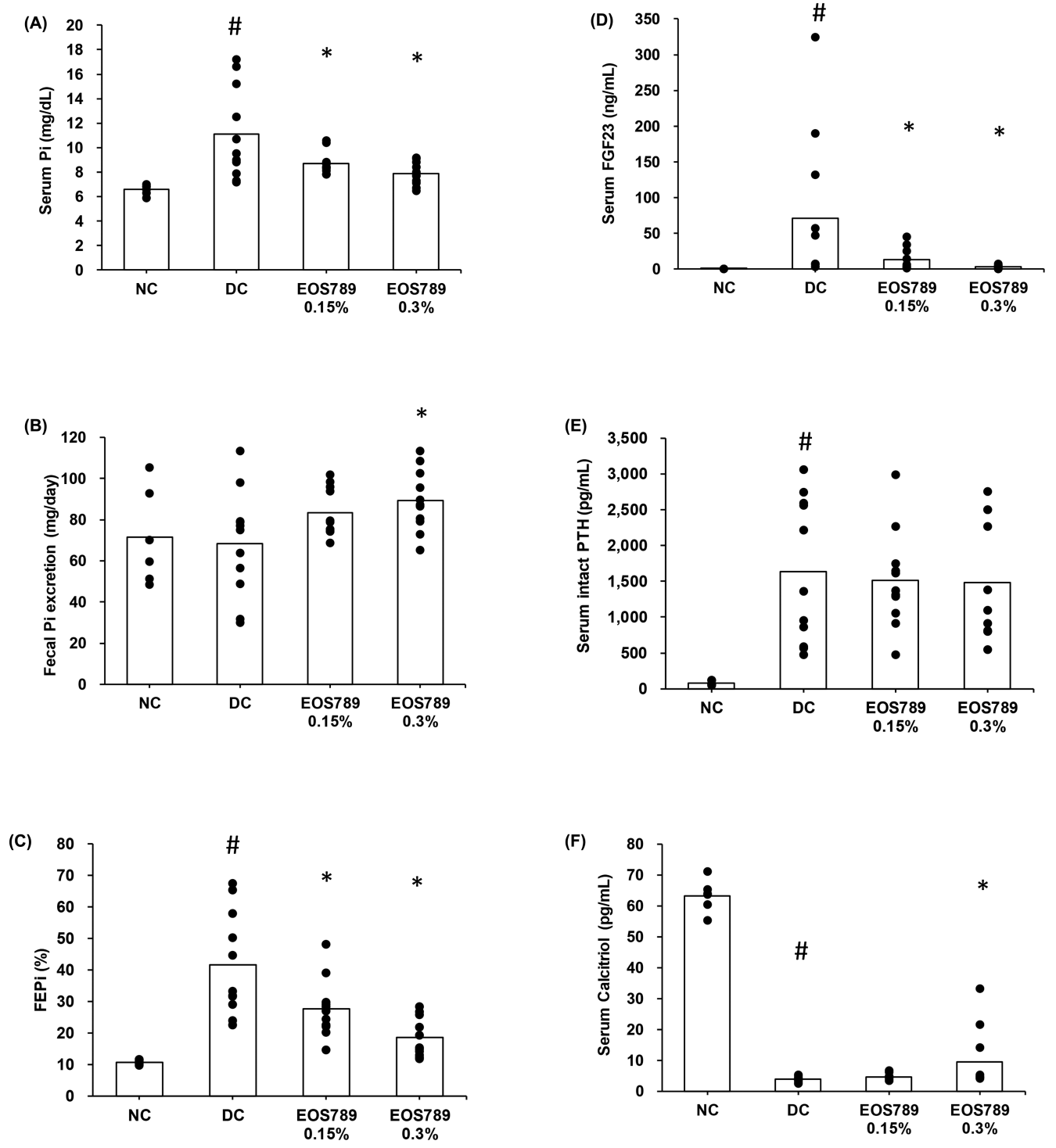

FIGURE 2 Effects of EOS789 on (A) serum Pi concentration, (B) fecal Pi excretion, (C) urinary fractional excretion of Pi, (D) serum FGF23 concentration, (E) serum intact PTH concentration, and (F) serum calcitriol concentration at the end of the study. Actual values are represented as dots, and columns represent means. NC, normal control; DC, disease control. #$p < .05$ significant difference between NC and DC (Welch test), *$p < .05$, significant difference from DC (parametric Williams test).

Mesangial cells are reported to accumulate in the extracellular matrix in hyperphosphatemia, which causes glomerular sclerosis.[16] Therefore, we evaluated the effects of EOS789 using the primary human mesangial cell line, hMes130hT. The high phosphate condition significantly increased the fibrotic markers in hMes130hT cells as was previously reported in immortalized human mesangial cells. Because they express PiT-1 and PiT-2, hMes130hT cells actively uptake phosphate in medium. EOS789 significantly suppressed the phosphate-mediated activation of the mesangial cells in a dose-dependent manner. EOS789, by inhibiting the active

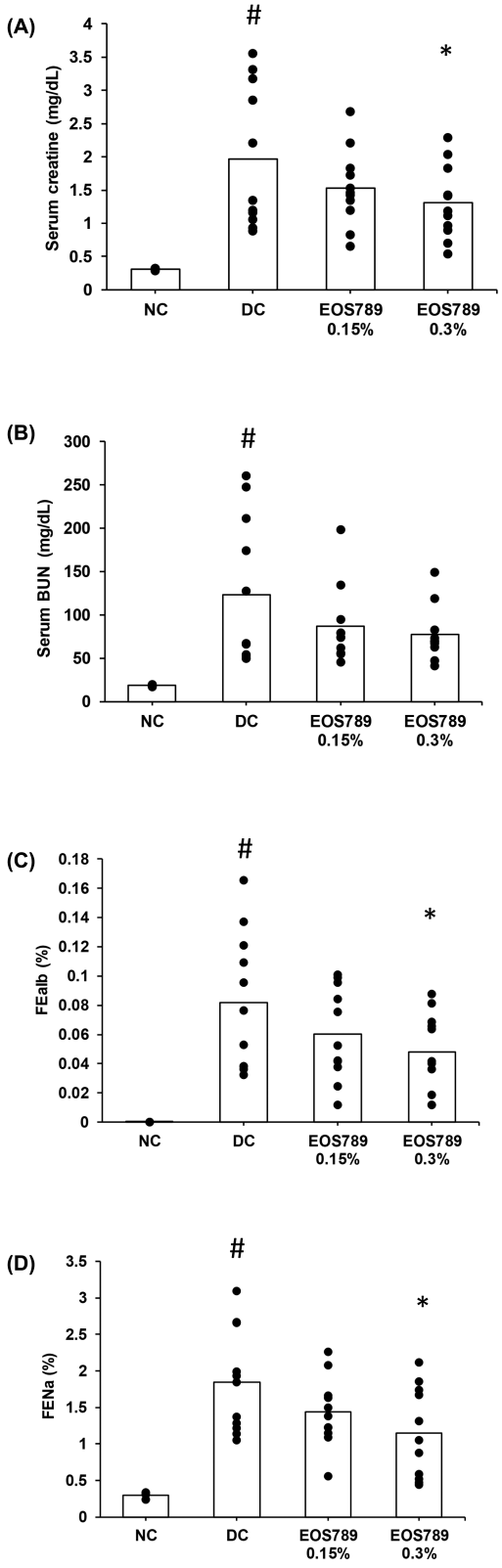

FIGURE 3 Effects of EOS789 on (A) serum creatinine concentration, (B) serum BUN concentration, (C) % of fractional excretion of albumin, and (D) % of fractional excretion of sodium at the end of the study. Actual values are represented as dots, and columns represent means. NC, normal control; DC, disease control. #$p < .05$ significant difference between NC and DC (Welch test), *$p < .05$, significant difference from DC (parametric Williams test).

phosphate uptake via PiT-1/2, suppressed the fibrotic change in hMes130hT cells. As EOS789 has the property of low absorption, the blood concentration of EOS789 was only 0.87 ± 0.08 μmol/L at 0.3% food admixture in the anti-GBM rats. Considering the protein binding of EOS789, this concentration might not be enough to inhibit PiT1/2.[18] Further experiments are needed to determine if EOS789 directly inhibit phosphate absorption in mesangial cells in vivo. Although the link between crescent formation and mesangial cell activation is not fully understood, they are correlated in human IgA nephropathy patients.[31] In addition, mesangial cell-specific deletion of connective tissue growth factor (CTGF) suppresses crescent formation in anti-GBM mice.[32] Taken together, this indicates that the inhibition of mesangial cells ameliorates crescent formation. EOS789 would inhibit the mesangial cell activation primarily by improving hyperphosphatemia, and additionally by inhibiting phosphate uptake in mesangial cells, leading to the amelioration of crescent formation.

Unexpectedly, EOS789 significantly reduced serum sodium and the urinary fractional excretion of sodium, indicating that EOS789 inhibited intestinal absorption of not only phosphate but also sodium. The Slc34a and Slc20a transporters inhibited by EOS789 are sodium-driven transporters. Therefore, EOS789 would also inhibit sodium absorption in small intestine. In fact, fecal sodium excretion was significantly increased by EOS789 0.3% food admixture compared with diseased control (0.71 ± 0.04 vs. 0.31 ± 0.07 mmol/day, $p < .05$). High sodium is thought to contribute to the progression of kidney disease,[33,34] therefore the restriction of intestinal sodium uptake by EOS789 would partly ameliorate kidney disease.

EOS789 dramatically reduced FGF23 by improving hyperphosphatemia. Interestingly, EOS789 significantly reduced serum PTH, but this effect is less obvious compared to other nephropathy models that suppressed serum FGF23.[18] Phosphate binders decrease both FGF23 and PTH to the same extent in CKD-induced hyperphosphatemia rats.[14,35,36] The difference may be due to the concentration of free phosphate in the intestinal lumen. Phosphate binders decrease the free phosphate, whereas EOS789, an inhibitor of phosphate transporters, increases the free phosphate. Berndt et al. reported that intestinal phosphate is a modulator of phosphate metabolism in rats.[37] Although acute increase of intestinal phosphate does not influence serum PTH in their report, chronic change in intestinal phosphate might influence phosphate metabolism including PTH secretion. In addition, free phosphate would bind calcium and might decrease calcium absorption. The precise mechanism of the difference between EOS789 and phosphate binders remains unknown and should be clarified.

Serum FGF23 is mainly regulated by serum phosphate; however, G3P has been recently reported to stimulate FGF23 production in bone.[27] Renal G3P is upregulated in ischemic-reperfusion injury mice and AKI patients after cardiac surgery. In the anti-GBM rats, we also found the significant increase of serum G3P. In this model ischemia would occur in glomeruli and/or tubules and would enhance the G3P synthesis. EOS789 might decrease the serum G3P by the amelioration of glomerular disease, which would partly contribute to a decrease in serum

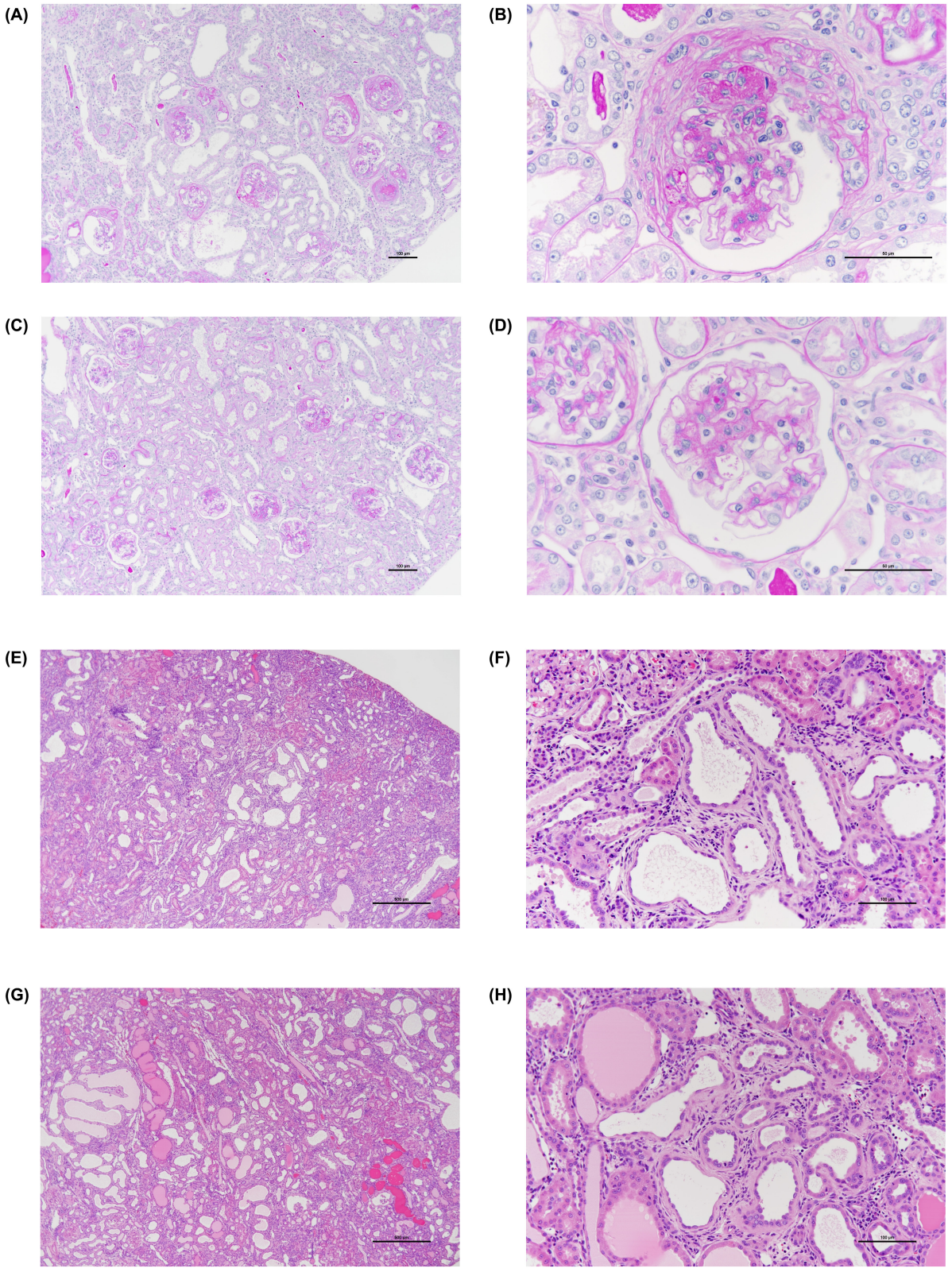

FIGURE 4 Effects of EOS789 on renal crescent formation and tubule-interstitial fibrosis. (A, B) renal crescent formation of disease control, (C, D) EOS789 0.3% with periodic acid-Schiff (PAS) staining, and (E, F) tubule-interstitial fibrosis of disease control, (G, H) EOS789 0.3% with hematoxyline-eosin (HE) staining. Bars =100 μm (A, C, F, H), 50 μm (B, D) and 500 μm (E, G).

FGF23. Apart from G3P, Egli-Spichtig et al reported that inflammation induces ectopic renal FGF23 expression in a mouse CKD model.[28] They suggested that TNFα may regulate renal FGF23 expression through NFκB-stimulating orphan nuclear receptor Nurr1 expression. In our report, we also observed the increase of FGF23, TNFα, and Nurr1 mRNA in the anti-GBM rats; however, EOS789 treatment did not decrease these

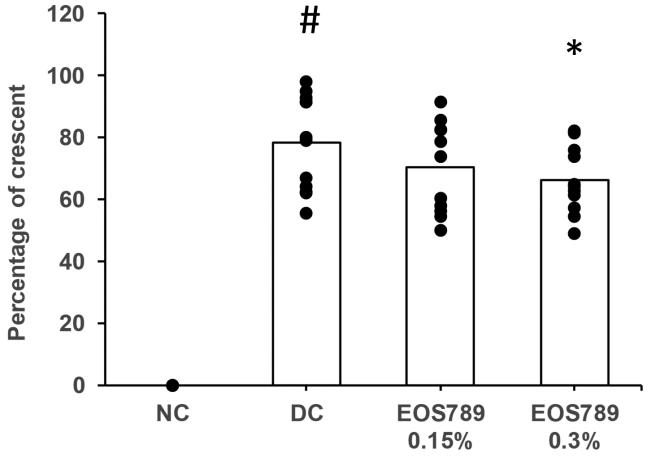

FIGURE 5 Effects of EOS789 on crescent formation. Percentage of crescentic glomeruli in total glomeruli at the end of the study. Actual values are represented as dots, and columns represent means. NC, normal control; DC, disease control. #$p < .05$ significant difference between NC and DC (Welch test), *$p < .05$, significant difference from DC (parametric Williams test).

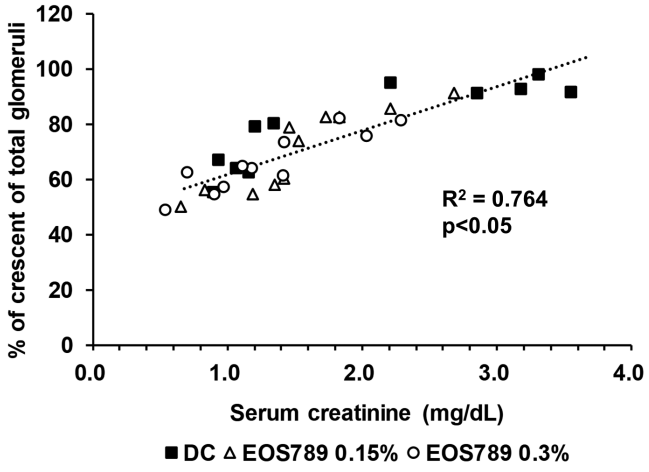

FIGURE 6 Correlation between crescent of formation and serum creatinine at the end of study. DC, disease control. The correlation was analysed by Spearman's rank correlation coefficient.

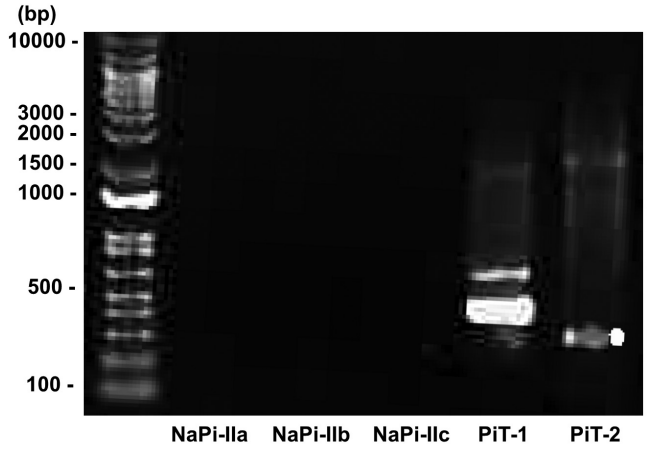

FIGURE 7 mRNA expression of NaPi-IIa, NaPi-IIb, NaPi-IIc, PiT-1, and Pit-2 in human mesangial cells.

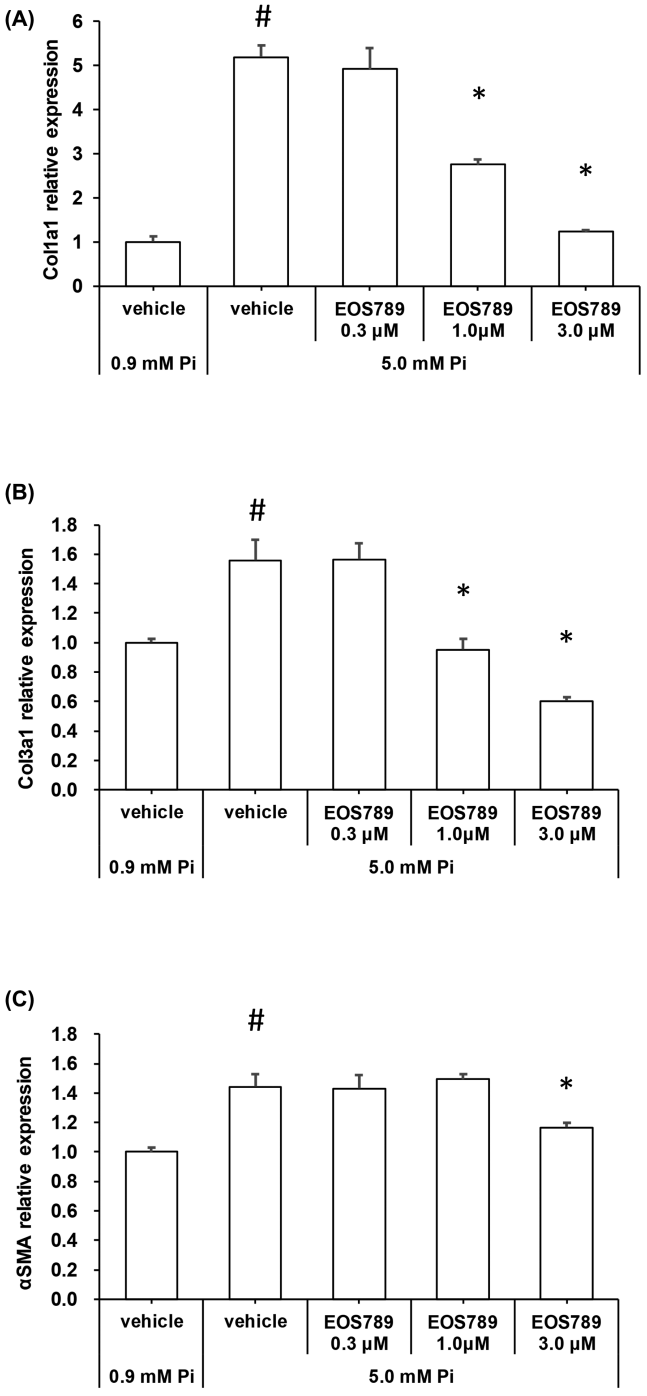

FIGURE 8 Relative gene expressions of (A) collagen type 1 α1 (Col1a1), (B) collagen type 3 α1 (Col3a1), and (D) α-smooth muscle actin (αSMA) in human mesangial cells. Values are expressed as mean +SE. #$p < .05$ significant difference between 0.9 mM Pi vehicle and 5.0 mM Pi vehicle (Welch test), *$p < .05$, significant difference from 5.0 mM Pi vehicle (parametric Williams test).

expressions. EOS789 would not affect the inflammatory-mediated renal FGF23 expression in anti-GBM rats.

In this study EOS789 ameliorated kidney disease progression in experimental anti-GBM nephritis rats. EOS789 would inhibit the mesangial cell activation primarily by improving hyperphosphatemia, and additionally by inhibiting phosphate uptake in mesangial cells,

leading to the amelioration of crescent formation. In conclusion EOS789 would not only be useful against hyperphosphatemia but may also have a potential to relieve mesangial proliferative glomerulonephritis with crescent formation.

Finally, this study has some limitations. Because the disease progression in the anti-GBM rats was much more rapid compared with that in clinical, the effect of EOS789 was only proved in the preventive dosing regimen. To show the therapeutic effects of EOS789, further study to treat with EOS789 when hyperphosphatemia has already occurred is needed. The interstitial fibrosis was not so obvious in the anti-GBM rats in this study that the effect of EOS789 for fibrosis could not be evaluated. Renal fibrotic model for example, unilateral ureter obstructive mice, should be used in near future study. In addition, the effects of EOS789 in this study was relatively mild and not remarkable. Other factors for example, proteinuria, hypertension, or inflammation play a much larger role in inducing disease progression than hyperphosphatemia in this model. Although EOS789 could ameliorate kidney disease progression that is due to hyperphosphatemia, it remains unclear if EOS789 could show the additive effects when used with renin-angiotensin system inhibitor, SGLT2 inhibitor, or immunosuppressant. Further study in combination with these drugs should be conducted to guess the effects of EOS789 in kidney disease progression in clinical.

AUTHOR CONTRIBUTIONS

Participated in research design: Tsuboi, Ichida, and Horiba. *Conducted experiments*: Ichida, Murai, Tsuboi, Maeda, Kato, Iida, Ohtomo, and Horiba. *Contributed new reagents or analytic tools*: Horiba. *Performed data analysis*: Tsuboi, Murai, Kato, and Horiba. *Wrote or contributed to the writing of the manuscript*: Tsuboi, Murai, and Horiba.

ACKNOWLEDGMENTS

We thank Masashi Yoshida and Mei Banba (Chugai Pharmaceutical Co., Ltd.) for technical assistance and Jacob Davis (Chugai Pharmaceutical Co., Ltd.) for his assistance with English usage. We also thank Prof. Jürgen Floege (RWTH Aachen University), Dr. Takei Kake and Dr. Nobuyuki Hori (Chugai Pharmaceutical Co., Ltd.) for their helpful discussion.

DISCLOSURE

The authors have declared that no conflicts interest exists.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

ORCID

Yoshinori Tsuboi https://orcid.org/0000-0001-9710-1833

REFERENCES

1. Fouque D, Pelletier S, Mafra D, Chauveau P. Nutrition and chronic kidney disease. *Kidney Int*. 2011;80:348-357.
2. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J Am Soc Nephrol*. 2004;15:2208-2218.
3. Kanbay M, Goldsmith D, Akcay A, Covia A. Phosphate-the silent stealthy cardiorenal culprit in all stages of chronic kidney disease. A systematic review. *Blood Purif*. 2009;27:220-230.
4. Palmer SC, Hayen A, Macaskill P, et al. Serum levels of phosphorus, parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease. a systematic review and meta-analysis. *JAMA*. 2011;305:1119-1127.
5. Tentori F, Blayney MJ, Albert JM, et al. Mortality risk for dialysis patients with different levels of serum calcium, phosphorus, and PTH. The dialysis outcomes and practice patterns study (DOPPS). *Am J Kidney Dis*. 2008;52:519-530.
6. Bleyer AJ, Burke SK, Dillon M, et al. A comparison of the calcium-free phosphate binder sevelamer hydrochloride with calcium acetate in the treatment of hyperphosphatemia in hemodialysis patients. *Am J Kidney Dis*. 1999;33:694-701.
7. Hutchison AJ, Wilson RJ, Garafola S, Copley JB. Lanthanum carbonate. Safety data after 10 years. *Nephrology*. 2016;21:987-994.
8. Pieper AK, Haffner D, Hoppe B, et al. A randomized crossover trial comparing sevelamer with calcium acetate in children with CKD. *Am J Kidney Dis*. 2006;47:625-635.
9. Sheikh MS, Maguire JA, Emmett M, et al. Reduction of dietary phosphorus absorption by phosphorus binders. A theoretical, in vitro, and in vivo study. *J Clin Invest*. 1989;83:66-73.
10. Floege J, Covic AC, Ketteler M,. et al. Long-term effects of the iron-based phosphate binder, sucroferric oxyhydroxide, in dialysis patients. *Nephrol Dial Transplant*. 2015;30:1037-1046.
11. Castañeda TR, Méndez M, Davison I, et al. The novel phosphate and bile acid sequestrant polymer SAR442357 delays disease progression in a rat model of diabetic nephropathy. *J Pharmacol Exp Ther*. 2021;376:190-203.
12. Jing W, Nunes ACF, Farzaneh T, Khazaeli M, Lau WL, Vaziri ND. Phosphate binder, ferric citrate, attenuates anemia, renal dysfunction, oxidative stress, inflammation, and fibrosis in 5/6 nephrectomized ckd rats. *J Pharmacol Exp Ther*. 2018;367:129-137.
13. Katai K, Iwamoto A, Kimura Y, et al. Wakame (Undaria pinnatifida) modulates hyperphosphatemia in a rat model of chronic renal failure. *J Med Invest*. 2015;62:68-74.
14. Maeda A, Fukushima N, Horiba N, Segawa H, Miyamoto KI. The role of extracellular phosphate levels on kidney disease progression in a podocyte injury mouse model. *Nephron*. 2019;142:135-146.
15. Nagano N, Miyata S, Obana S, et al. Sevelamer hydrochloride, a phosphate binder, protects against deterioration of renal function in rats with progressive chronic renal insufficiency. *Nephrol Dial Transplant*. 2003;18:2014-2023.
16. Papadimitriou A, Peixoto EB, Silva KC, Lopes de Faria JM, Lopes de Faria JB. Inactivation of AMPK mediates high phosphate-induced extracellular matrix accumulation via NOX4/TGFss-1 signaling in human mesangial cells. *Cell Physiol Biochem*. 2014;34:1260-1272.
17. Tan X, Xu X, Zeisberg M, Zeisberg EM. DNMT1 and HDAC2 cooperate to facilitate aberrant promoter methylation in inorganic phosphate-induced endothelial-mesenchymal transition. *PLoS One*. 2016;11:e0147816.
18. Tsuboi Y, Ohtomo S, Ichida Y, et al. EOS789, a novel pan-phosphate transporter inhibitor, is effective for the treatment of chronic kidney disease-mineral bone disorder. *Kidney Int*. 2020;98:343-354.
19. Hill Gallant KM, Stremke ER, Trevino LL, et al. EOS789, a broad-spectrum inhibitor of phosphate transport, is safe with an indication of efficacy in a phase 1b randomized crossover trial in hemodialysis patients. *Kidney Int*. 2021;99:1225-1233.
20. Ito M, Yamada H, Okamoto K, Suzuki Y. Crescentic type nephritis induced by anti-glomerular basement membrane (GBM) serum in rats. *Jpn J Pharmacol*. 1983;33:1145-1154.

21. Ohtake Y, Okamoto N, Ono Y, et al. Inventors, Chugai Pharmaceutical Co., Ltd., assignee. Dihydropyridazine-3,5-dione derivative and pharmaceuticals containing the same. U.S. patent 2016/0002251 Al, 2016.
22. Katsumata K, Kusano K, Hirata M, et al. Sevelamer hydrochloride prevents ectopic calcification and renal osteodystrophy in chronic renal failure rats. *Kidney Int*. 2003;64:441-450.
23. Matsuo A, Iida A, Tanimoto M, Matsushita M, Miyamoto K. The utility of the phosphate binder, ferric citrate hydrate (JTT-751), about phosphorus absorption-reducing effect in normal rats. *Ren Fail*. 2014;36:1291-1297.
24. Jat PS, Sharp PA. Cell lines established by a temperature-sensitive simian virus 40 large-T-antigen gene are growth restricted at the nonpermissive temperature. *Mol Cell Biol*. 1989;9:1672-1681.
25. Mizuno K, Tai H, Yuuki M. (2012) Inventors, Chugai Pharmaceutical Co., Ltd., assignee. Immortalized human mesangial Cells. JP patent 501624B2, 2012.
26. Harding SD, Sharman JL, Faccenda E, et al. The IUPHAR/BPS Guide to PHARMACOLOGY in 2019: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucleic Acids Res*. 2018;46:D1091-1106. doi:10.1093/nar/gkx1121
27. Simic P, Kim W, Zhou W, et al. Glycerol-3-phosphate is an FGF23 regulator derived from the injured kidney. *J Clin Invest*. 2020;130:1513-1526.
28. Egli-Spichtig D, Imenez Silva PH, Glaudemans B, et al. Tumor necrosis factor stimulates fibroblast growth factor 23 levels in chronic kidney disease and non-renal inflammation. *Kidney Int*. 2019;96:890-905.
29. McEvoy AN, Murphy EA, Ponnio T, et al. Activation of nuclear orphan receptor NURR1 transcription by NF-kappa B and cyclic adenosine 5'-monophosphate response element-binding protein in rheumatoid arthritis synovial tissue. *J Immunol*. 2002;168:2979-2987.
30. Meir T, Durlacher K, Pan Z, et al. Parathyroid hormone activates the orphan nuclear receptor Nurr1 to induce FGF23 transcription. *Kidney Int*. 2014;86:1106-1115.
31. Zhang X, Shi S, Ouyang Y, et al. A validation study of crescents in predicting ESRD in patients with IgA nephropathy. *J Transl Med*. 2018;16:115.
32. Toda N, Mori K, Kasahara M, et al. Crucial role of mesangial cell-derived connective tissue growth factor in a mouse model of anti-glomerular basement membrane glomerulonephritis. *Sci Rep*. 2017;7:42114.
33. Krikken JA, Laverman GD, Navis G. Benefits of dietary sodium restriction in the management of chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2009;18:531-538.
34. Vegter S, Perna A, Postma MJ, Navis G, Remuzzi G, Ruggenenti P. Sodium intake, ACE inhibition, and progression to ESRD. *J Am Soc Nephrol*. 2012;23:165-173.
35. Nagano N, Miyata S, Abe M, et al. Effect of manipulating serum phosphorus with phosphate binder on circulating PTH and FGF23 in renal failure rats. *Kidney Int*. 2006;69:531-537.
36. Phan O, Maillard M, Malluche HH, Stehle JC, Funk F, Burnier M. Effects of sucroferric oxyhydroxide compared to lanthanum carbonate and sevelamer carbonate on phosphate homeostasis and vascular calcifications in a rat model of chronic kidney failure. *Biomed Res Int*. 2015;2015:515606.
37. Berndt T, Thomas LF, Craig TA, et al. Evidence for a signaling axis by which intestinal phosphate rapidly modulates renal phosphate reabsorption. *Proc Natl Acad Sci USA*. 2007;104:11085-11090.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Tsuboi Y, Ichida Y, Murai A, et al. EOS789, pan-phosphate transporter inhibitor, ameliorates the progression of kidney injury in anti-GBM-induced glomerulonephritis rats. *Pharmacol Res Perspect*. 2022;10:e00973. doi:10.1002/prp2.973