Development and Validation of a Stem Cell–Based Kidney Organoid Platform for Nephrotoxicity Assessment

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Abstract

Considerable efforts have been made to reduce animal experimentation in accordance with the 3R principles; replacement, reduction, and refinement; thus New Approach Methodologies have emerged as an urgent necessity. In this context, we developed a kidney organoid system derived from adult rat kidney stem (KS) cells and evaluated its potential as a screening platform for nephrotoxicity. Nephrotoxicity was assessed in KS cell-derived organoids treated with known nephrotoxic agents, including cisplatin and gentamicin. Additionally, we investigated the nephrotoxicity of puberulic acid, a compound associated with acute kidney injury linked to the *Beni-koji CholesteHelp* supplement recently reported in Japan, using both kidney organoids and wild-type mice. The organoid model successfully recapitulated morphological injury induced by known nephrotoxins and enabled quantitative assessment via Kidney Injury Molecule-1 (Kim-1) mRNA expression. In organoids, puberulic acid induced apoptosis, demonstrated by increased cleaved caspase-3–positive cells, similar to cisplatin-treated samples. Transmission electron microscopy revealed structural alterations such as loss of intercellular tight junctions and vacuolization, consistent with acute tubular necrosis (ATN). Quantitative real-time PCR analysis confirmed significant Kim-1 upregulation following nephrotoxic exposure. *In vivo*, puberulic acid–treated mice exhibited impaired renal function and histological findings of ATN, closely resembling the organoid results. This KS cell-derived kidney organoid system offers a reproducible and rapid screening tool for nephrotoxicity with simple protocol. The ability to assess both morphological and quantitative changes following drug exposure highlights the utility of this organoid platform for toxicological evaluation and supports its potential as an alternative to animal models.

Introduction

Considerable efforts have been made to reduce animal experimentation in accordance with the 3R principles: replacement, reduction, and refinement, and New Approach Methodologies (NAMs) have emerged an urgent necessity [1]. The development of nephrotoxicity assessment models using kidney organoids derived from induced pluripotent stem (iPS) cells has been advancing [2 3], attracting attention as a promising alternative to animal testing. As an alternative *in vitro* approach for nephrotoxicity assessment, we have investigated the use of kidney stem (KS) cells derived from a stem/progenitor-like cell line from the renal proximal tubule S3 segment of adult rats. These KS cells have been shown to differentiate into proximal tubules and distal tubules in a three-dimensional culture system [4], and this organoid model has previously been demonstrated to recapitulate tubular toxicity in response to cisplatin exposure at the pathological level [5].

We recently reported that specific lot of *Beni-koji CholesteHelp* supplement induced pathological renal tubule injury in this kidney organoid model [6]. This supplement, intended for lowering plasma cholesterol levels, was implicated in multiple cases of acute kidney injury (AKI) in patients who consumed it in Japan during late 2023 and early 2024 [7-10]. Contamination with puberulic acid has been suggested as a potential cause [11]; however, its nephrotoxic effects remains to be explored. In the present study, we established a refined nephrotoxicity assessment model using this kidney organoid, enabling quantitative evaluation of tubular injury through Kidney Injury Molecule-1 (Kim-1) expression measured by quantitative real-time PCR (qPCR). Furthermore, we applied this organoid-based model to investigate the nephrotoxicity of puberulic acid.

Methods

Cell culture and organoid formation

The KS cells were maintained on type I collagen (IWAKI AGC Techno Glass Corporation, Japan) under previously described undifferentiated culture conditions [12]. The KS cell-derived organoids were generated as previously described [4], The cells were trypsinized, harvested, and resuspended in medium, ensuring minimal residual trypsin to avoid interference with cell aggregation. Cell suspensions were seeded onto the plates by hanging drop method, with 2.0×10^5 cells in each 25-30 µL droplet per well. The clusters were incubated in 5% CO_2 and 100% humidity at 37°C for 8 hours. For three-dimensional culture, 200 µL of a half-Matrigel solution, consisting of a 1:1 mixture of Matrigel and differentiation medium (DMEM/F-12 supplemented with 10% fetal bovine serum (FBS), 250 ng/mL hepatocyte growth factor (HGF), 250 ng/mL glial cell line-derived neurotrophic factor (GDNF), 250 ng/mL basic fibroblast growth factor (bFGF), 250 ng/mL epidermal growth factor (EGF), and 250 ng/mL bone morphogenetic protein-7 (BMP-7), was applied to Transwell inserts (Corning Life Sciences, USA) and placed into 24-well plates containing 400 µL of differentiation medium. KS cell clusters were transferred into the half-Matrigel solution and cultured at 37°C with 5% CO_2 and 100% humidity for 2-3 weeks. Cisplatin (60 µM; FUJIFILM Wako Pure Chemical Corporation), gentamicin (10 mM; Selleck Chemicals, USA), and puberulic acid (2, 10 and 50 µM) were applied to the organoids, and were cultured in differentiation medium for 72 hours.

Animal experiment

The experimental protocol was approved by the Animal Ethics Review Committee of the Okayama University Graduate School of Medicine, Density and Pharmaceutical Sciences (OKU-2024474). Eight-week-old male C57BL/6N mice (CLEA Japan, Japan) were housed with free access to tap water and standard laboratory chow. All animals were cared for in accordance with the relevant guidelines and regulations. Mice were randomly assigned to the following two groups (n = 6 per group): the control group (CTR) and the puberulic acid group (PUB). Mice in the PUB group were intraperitoneally injected with puberulic acid (5.0 mg/kg body weight) on days 0 and 1. Mice in the CTR group were intraperitoneally injected with 100 µL saline on days 0 and 1. All mice were euthanized on day 4, and blood and kidneys samples were harvested for analysis.

Blood and Urine Biochemical Measurements

Mouse blood samples were collected immediately before euthanizing, and serum creatinine and Blood urea nitrogen (BUN) were measured at the Department of Animal Resources, Advanced Science Research Center, Okayama University. Mouse urine samples were centrifuged to remove cells, cell debris, and other contaminants. Urinary albumin was measured with the Mouse Urinary Albumin Assay Kit (FUJIFILM Wako Pure Chemical Corporation, Japan), and urinary creatinine was measured with a creatinine kit (FUJIFILM Wako Pure Chemical Corporation, Japan).

Histological and immunofluorescence analyses

Organoids were fixed with 10% formalin (Nacalai Tesque, Japan), embedded in paraffin, sectioned at 4 µm, and stained with periodic acid-Schiff (PAS). Tubular damage was scored by calculation of the percentage of tubules in the corticomedullary junction that displayed tubular dilatation, tubular atrophy, tubular cast formation, and sloughing of tubular epithelial cells or loss of the brush border, and: 0, none; 1, ≤10%; 2, 11%–25%; 3, 26%–45%; 4, 46%–75%; –5, >76%. At least 10 high-power fields (magnification, ×200) per section for each sample were examined (n=6 per group) [13, 14]. For immunofluorescence, deparaffinized sections underwent antigen retrieval with EDTA, blocked with 1% BSA-PBS, and incubated with primary antibodies [fluorescein-labeled lotus tetragonolobus lectin (LTL) (Invitrogen), rabbit cleaved caspase-3 (Cell Signaling Technology, USA), and goat Kim-1 (R&D systems, USA) as previously described [15]. 4',6'-diamidino-2-phenylindole (DAPI) (Roche Diagnostic, Switzerland) counterstain were used to visualize nuclei. Imaging was performed with FSX-100 (Olympus, Japan). Cleaved-caspase 3-positive cells per field were evaluated in three kidney organoids tissue sections using five randomly selected images at x200 magnification.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) analysis was conducted as described previously [4]. Specimens were fixed in 0.1 M cacodylate buffer containing 2.5% glutaraldehyde (pH 7.2) and processed thin sections were stained with uranyl acetate and citrate and examined using a Hitachi H-700 electron microscope.

Western Blotting Analysis

Western blotting analysis was performed as previously described [16]. Total proteins were extracted using RIPA buffer (Thermo Fisher Scientific, USA) supplemented with protease inhibitors (Promega, USA). Protein concentration was determined using a BCA Protein Assay Kit (Thermo Fisher Scientific, USA). Equal

amounts of protein were separated by SDS-PAGE, transferred to nitrocellulose membranes, and blocked with 5% skim milk. Membranes were incubated overnight at 4°C with primary antibodies [GAPDH (Cell Signaling Technology, USA), Kim-1 (R&D systems, USA)], followed by HRP-conjugated secondary antibodies. Signal detection was performed with an enhanced chemiluminescence system (GE Healthcare, USA) and imaged using the Amersham Imager 600. Densitometric analysis was performed using ImageJ software.

Real-time RT-PCR

RNA isolation and qPCR were performed using the kidney organoids (n = 3 per group), as previously described [17]. Total RNA was extracted using an RNA extraction kit (Qiagen Sciences, USA). Real-time PCR was performed on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA) using TaqMan® Fast Advanced Master Mix (Applied Biosystems, USA). The amplification protocol included an initial enzyme activation at 95 °C for 20 seconds, followed by 40 cycles of denaturation at 95 °C for 1 second and annealing at 60 °C for 20 seconds. The comparative threshold cycle (Ct) method was used to calculate fold amplification. Gene expression was analyzed via qPCR using primers specific for Kim-1(assay ID: Rn00597703_m, Thermo Fisher Scientific, USA). Controls were consistently detected to confirm the validity of the qPCR assay.

Cell viability analysis

An immortalized human proximal tubular epithelial cell line, HK-2 (American Type Culture Collection, USA), cultured in DMEM/F12 supplemented with 10% FBS, were seeded in 96-well plates and incubated overnight at a density of 5,000 cells per well in 100 μL of medium to adhere. Then, puberulic acid was added at various concentrations (0.4-50 μM) and incubated for 24 hours. The cell viability following puberulic acid treatment was assessed using Cell counting kit-8, according to the manufacturer's protocol (Dojindo Laboratories, Japan), as previously described [18]. Absorbance at 450 nm was measured using a microplate reader (Thermo Fisher Scientific, USA), and cell viability was expressed as a percentage relative to the control group (n=8 per group).

Statistical analysis

Data are expressed as means ± SD. Statistical analysis was performed using JMP software (JMP® 13.2, SAS Institute). For comparisons between two groups, either the Student's t-test or the Wilcoxon rank-sum test was used. For comparisons among three

or more groups, One-way ANOVA with a Tukey's post hoc test was employed. $P < 0.05$ was considered as significantly different.

Results

Establishment of nephrotoxicity model based on kidney organoids.

Kidney organoids were generated using KS cells (Figure 1A), and the tubular injury was evaluated following treatment with the known nephrotoxicants, cisplatin and gentamicin. Histological evaluation by stereomicroscopy revealed detachment of tubules cells, tubular narrowing and disruption in the cisplatin-treated group at 72 hours. Hematoxylin and eosin (H&E) staining at 72 hours further confirmed detachment of tubular cells as well as cellular and nuclear degeneration in the cisplatin and gentamicin group (Figure 1B). Immunofluorescence staining demonstrated increased expression of Kim-1, a marker of renal proximal tubule injury, in renal tubular cells and within the lumen of the renal tubules in the cisplatin and gentamicin group, compared to the control group (Figure 1C). The qPCR analysis corroborated these findings, showing significantly upregulated Kim-1 expression in both treatment groups compared to the control group (Figure 1D). In addition, immunofluorescence analysis showed a significant increase in cleaved caspase-3-positive apoptotic cells in the tubules of kidney organoids treated organoids (Figure 1, C and E). Collectively, these findings indicate that cisplatin and gentamicin recapitulate key features of proximal tubule injury in this KS cell–derived kidney organoids.

Puberulic Acid Induces Kidney Organoid Damage

To validate the established kidney organoid model for nephrotoxicity assessment, we investigated the effects of puberulic acid, a suspected causative agent in the recent supplement-induced AKI cases reported in Japan. Initially, a cytotoxicity assay was performed on HK-2 cells using various concentrations of puberulic acid (Supplemental Figure 1). HK-2 cell viability significantly decreased at 50 μM, suggesting the potential nephrotoxic effects. Subsequently, we applied the kidney organoid model to further evaluate the nephrotoxicity of puberulic acid. Histological evaluation by stereomicroscopy revealed detachment of tubules cells in organoids treated with 50 μM puberulic acid (Figure. 2A). H&E staining corroborated this finding, showing clear evidence of tubular epithelial detachment at the same concentration (Figure. 2B). These

changes were not evident in organoids treated with 10 μM puberulic acid at or untreated controls. TEM analysis further highlighted ultrastructural abnormalities in organoids treated with both 10 and 50 μM puberulic acid (Figure 2C), including separation of renal tubular cells due to loss of intercellular junctions and the presence of low-electron-density vesicles containing remnants of organelles within dilated vacuoles, likely representing lysosomes. In contrast, organoids in the control groups maintained intact tubular structure. These morphological changes were consistent with findings previously reported in kidney organoids treated with *Beni-koji Cholestehelp* supplement [6] and resemble features of drug-induced acute tubular necrosis observed in human kidneys. In addition, immunofluorescence staining demonstrated upregulation of Kim-1 expression in renal tubular cells and within the lumen of the renal tubules in organoids treated with puberulic acid (Figure. 2D). Western blotting and qPCR analysis confirmed an increase in Kim-1 expression in puberulic acid-treated organoids compared to the control group (Figure. 2, E and F). Additionally, cleaved caspase-3-positive apoptotic cells were significantly increased in the tubular regions of puberulic acid-treated organoids, as revealed by immunofluorescence (Figure 2, D and G). Collectively, these results indicate that puberulic acid induces proximal tubular injury and apoptosis, supporting its nephrotoxic potential.

Puberulic Acid Induces Acute Kidney Injury in Mice

To validate the nephrotoxicity effects of puberulic acid observed in the kidney organoid system, we further conducted *in vivo* experiments. Mice with puberulic acid injection showed increased serum creatinine levels (Figure 3A). BUN levels were also elevated in the puberulic acid-treated group, while the difference was not statistically significant (Figure 3B). The urinary albumin-to-creatinine ratio was significantly elevated in the PUB group ($p<0.01$) (Figure. 3C). PAS staining of kidney tissues in the puberulic acid-treated group showed tubular atrophy, shedding, and cast formation, suggestive of acute tubular necrosis, while no glomerular abnormalities were evident (Figure. 3D). The renal tubular injury score was significantly higher in the puberulic acid-treated group compared to the control group (Figure. 3E). TEM analysis showed vacuolar degeneration of proximal tubule cells, loss of the brush border, a reduction in mitochondria, and necrosis of renal tubular epithelial cells in the puberulic acid-treated group (Figure 3D). On the other hand, no ultrastructural abnormalities were detected in the glomeruli, distal tubules, or collecting ducts (Supplemental Figure. 2), suggesting that the effect of puberulic acid may be specific for proximal tubular cells. Immunofluorescence staining

revealed increased expression of Kim-1 and cleaved caspase-3 in the LTL-positive proximal tubular cells and within the lumen of the renal tubules in the puberulic acid-treated organoids, compared to the control group (Figure. 3F). Western blotting analysis showed that Kim-1 expression was significantly increased upregulated in the puberulic acid-treated group compared to the control group ($P<0.01$) (Figure. 3G).

Discussion

In the present study, we developed a nephrotoxicity assessment system using stem cell-based kidney organoids, where a quantitative analysis was possible using Kim-1 qPCR. We previously established KS cell-derived kidney organoids [11] and reported the utility as a screening platform for nephrotoxicity by evaluating morphological changes in the organoids following drug exposure [6]. The kidney organoids were treated with known nephrotoxic agents, such as cisplatin or gentamicin, and nephrotoxicity quantitatively evaluated using qPCR analysis of Kim-1, a biomarker highly upregulated in proximal tubules following acute kidney injury [19]. The expression of Kim-1 was significantly increased in the kidney organoids treated with nephrotoxic agents compared to the control group. The ability to quantitatively evaluate nephrotoxicity via qPCR further enhances the utility of the kidney organoids as a nephrotoxicity assessment platform.

In a previous study using iPS cell-derived kidney organoids, immunostaining and real-time PCR analysis confirmed an upregulation of Kim-1 expression in the proximal tubules of renal organoids following gentamicin treatment[20]. Furthermore, advances in automated kidney organoid culture using micro-well plates are reported to enable high-throughput screening (HTS), further broadening the applications of this technology in drug discovery, toxicity testing and selection of nephroprotective agents [2 3]. Although KS cell-derived kidney organoids are less differentiated and structurally complex compared to iPS cell-derived kidney organoids, this KS cell-derived kidney organoid system offers a cost-effective, reproducible, and rapid screening tool. This system has the potential to bridge the gap between conventional cell culture and animal models, whether in drug-induced toxicity or pre-clinical drug development.

AKI associated with *Beni-koji CholesteHelp* supplements, containing red yeast rice (Beni-Koji), emerged as a significant public health concern in Japan. In a study involving 102 patients who had taken *Beni-koji Choleste Help*, light microscopy revealed predominant tubulointerstitial changes, with tubulointerstitial nephritis in 50% of cases and tubular necrosis in 32%, while glomerular changes were less prominent [21]. We have previously reported that specific batches of *Beni-koji CholesteHelp supplements* directly

induced renal tubular injury using this kidney organoid model [6]. A recent investigation conducted by the National Institute of Health Sciences of Japan and its collaborators identified that the implicated product batches contained puberulic acid [22]. Further analysis confirmed that this compound can be synthesized by *Penicillium adamezioides*, a blue mold detected in the production facility of *Beni-koji*. In a 7-day repeated-dose toxicity study in rats, puberulic acid induced degeneration and necrosis of proximal tubules cells. These findings suggest that contamination by *P. adamezioides* dwelling in the factory may have occurred during the fermentation stage, resulting in puberulic acid production and subsequent renal toxicity. In the previous reports, puberulic acid has been isolated from culture broths of *Penicillium* species and shown to exhibit potent and selective antimalarial activity both *in vitro* and *in vivo* [23]. While puberulic acid exhibits toxicity *in vivo*, with four out of five mice dying by day 3 after receiving a dose of 5 mg/kg administered twice (on days 0 and 1) [24], its renal effects of puberulic acid have not been understood. In this study, we demonstrated that puberulic acid induced Kim-1 and cleaved caspase-3 expression in kidney organoids, and TEM findings highlighted structural and cellular damage in tubular regions, consistent with acute tubular necrosis observed in human kidneys. In mice treated with puberulic acid, upregulation of Kim-1 and the localization of cleaved caspase-3 in LTL-positive tubule were demonstrated, and TEM analysis revealed tubular necrosis in the proximal tubules. These findings are consistent with the upregulation of Kim-1 detected by qPCR and the increased expression of cleaved caspase-3 in tubular cells, further supporting the utility of the kidney organoid system for nephrotoxicity evaluation. The kidney organoids closely recapitulated the tubular injury observed in the mouse model, suggesting that they may serve as an alternative to mouse experiments in assessing the nephrotoxicity of drugs.

There are several limitations to this kidney organoid system. First, these kidney organoids lack the expression of several transporters, including OCT1, OCT2, OAT1, and OAT3 [5, 6]. As a result, drugs may have penetrated cells via passive diffusion rather than transporter-mediated uptake, which may affect the assessment of nephrotoxicity. Second, the absence of immune cells, blood vessels, and endothelial components limits the ability to assess kidney damage related to these factors in the kidney, such as tubulointerstitial nephritis, vascular injury, endothelial damage, and ischemia. Third, since toxins are applied externally rather than through physiological routes such as blood circulation and glomerular filtration, direct extrapolation of drug concentrations to clinical settings is not feasible. In spite of these limitations, the kidney organoid system remains a valuable *in vitro* tool for detecting direct tubular toxicity in a controlled environment. It offers a

reproducible, scalable, and ethically favorable alternative to animal models, and can be particularly useful in the early-phase nephrotoxicity screening.

In conclusion, this KS cell-derived organoid system demonstrates significant potential as a platform for nephrotoxicity testing and pre-clinical drug development. The concordance between findings from organoid and animal models highlights the reliability of this system as an *in vitro* tool for nephrotoxicity screening. Future studies should focus on further validation and refinement for clinical applications.

Disclosure

All authors declared no competing interests.

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Figure legends

Figure 1. Tubular Injury in Kidney Organoids Induced by Cisplatin and Gentamicin.

(A) Time-course Changes in Kidney Organoids following Cisplatin or Gentamicin. Time-course morphological changes of kidney organoids treated with cisplatin or gentamicin. Kidney organoids were cultured under control conditions (CTR) or treated with cisplatin (CIS) or gentamicin (GEN), and observed at 0, 24, and 72 hours. Morphological changes such as detachment of tubules cells, tubular narrowing and disruption became evident over time in CIS group, particularly after 72 hours. Enlarged images on the far right highlight the structural abnormalities. Scale bar, 1 mm. (B-E) Histological and immunofluorescence analysis of kidney organoids following treatment with cisplatin or gentamicin. (B) Hematoxylin and eosin (H&E) staining of kidney organoids untreated (CTR), treated with cisplatin (CIS), or gentamicin (GEN) for 72 hours. Tubular epithelial cell detachment (black arrows) and degeneration (red arrows) were observed in the CIS and GEN groups. (C) Immunofluorescence staining for kidney injury molecule-1 (Kim-1, red) and cleaved caspase-3 (red) with Lotus tetragonolobus lectin (LTL, green) and DAPI (blue). Increased Kim-1 and cleaved caspase-3 expression were observed in the CIS and GEN groups compared to the control. Scale bars, 20 μm. (D) Quantitative real-time PCR analysis showing significantly increased Kim-1 mRNA expression in the CIS and GEN group. Gene expression was normalized to Gapdh and expressed as fold change relative to the CTR group (n = 3 per group). (E) Quantification of cleaved caspase-3 positive apoptotic cells per field. Three independent samples were analysed for each group, with measurements taken from five randomly selected fields per sample. Data are presented as mean ± SEM. Analysis was conducted by Tukey's test. ** $P < 0.01$.

Figure 2. Tubular Injury in Kidney Organoids Induced by Puberulic Acid.

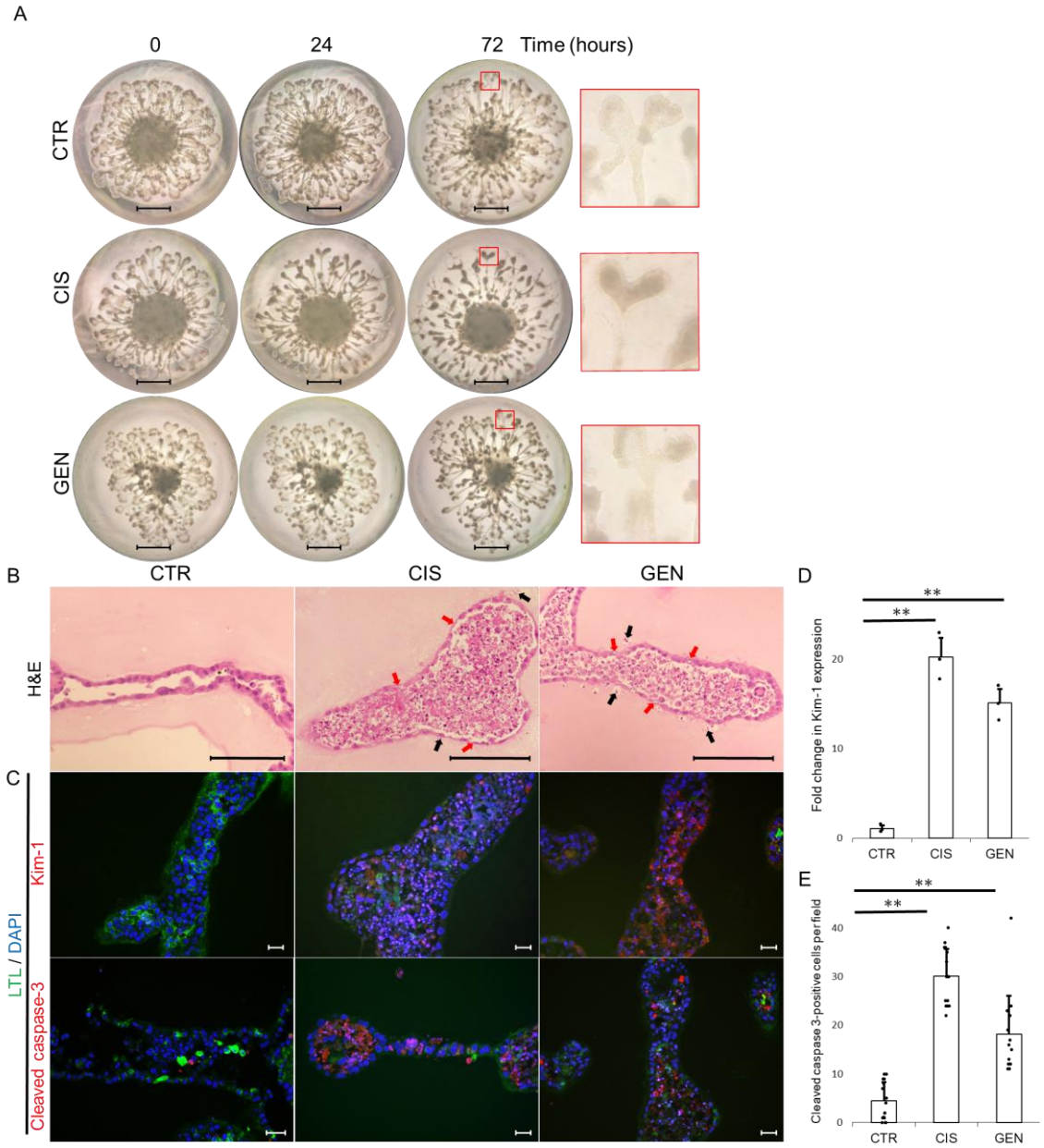
(A) Stereomicroscopy images of kidney organoids untreated (CTR) or puberulic acid (10 μM and 50 μM) for 72 hours. Detachment of tubules cells were observed in the PUB group (50 μM). Scale bars: 1 mm. (B) Hematoxylin and eosin (H&E) staining showing detachment of tubular cells (black arrows) and degeneration (red arrows), in the PUB group. Scale bars: 100 μm. (C) Transmission electron microscopy (TEM) images revealing separation of tubular cells (yellow arrows) and vacuolation (arrowheads) in the PUB groups. Scale bars: 10 μm. (D) Immunofluorescence staining for Kidney Injury Molecule-1 (Kim-1, red) and cleaved caspase-3 (red) in Lotus tetragonolobus lectin (LTL, green)-positive tubules, with nuclei counterstained by DAPI (blue) in kidney organoids untreated (CTR) or puberulic acid (10 μM and 50 μM) for 72 hours. Increased Kim-1

expression and apoptotic cells were observed in the PUB-treated group compared to the CTR group. Scale bars: 20 μm. (E) Western blot analysis of the kidney organoids showing upregulation of Kim-1 expression in the PUB group compared to the CTR group. (F) Quantification of cleaved caspase-3 positive apoptotic cells per field. Three independent samples were analyzed for each group, with measurements taken from five randomly selected fields per sample. Data are presented as mean ± SEM. (G) Quantitative real-time PCR showing significantly increased Kim-1 mRNA expression in the PUB (10, 50μM), compared to the CTR group. Gene expression was normalized to Gapdh and expressed as fold change relative to the CTR group. Data are presented as mean ± SEM (n = 3 per group). Analysis was conducted by Tukey's test. ** $P < 0.01$.

Figure 3. Puberulic Acid Induces Acute Kidney Injury in Mice.

Histological and functional evaluation in mice under control conditions (CTR) or treated with puberulic acid (PUB) after 4 days (n = 6 per group). (A) Serum creatinine (S-Cr) levels were significantly elevated in the PUB group compared to the CTR group. (B) Blood urea nitrogen (BUN) levels were elevated in the PUB group but without statistical significance. (C) Urinary albumin-to-creatinine ratio (U-albumin /Cr) was significantly increased in the PUB group compared to the CTR group. (D) Upper panels: Periodic acid–Schiff (PAS) staining; lower row: transmission electron microscopy (TEM) images of kidney section showing features consistent with acute tubular necrosis in the PUB group compared to the CTR group. Scale bars: 100 μm. (E) Tubular injury score was significantly increased in the PUB group compared to the CTR group. (F) Immunofluorescence staining of kidney sections showing increased expression of kidney injury molecule-1 (Kim-1, red) and cleaved caspase-3 (red) in the PUB-treated group compared to the CTR group. Nuclei were counterstained with DAPI (blue). Scale bars: 100 μm. (G) Western blot analysis demonstrating upregulation of Kim-1 protein expression in the PUB-treated group compared to the CTR group. Data are presented as mean ± SEM. Analysis was conducted by Student's t-test or Wilcoxon rank-sum test. * $P < 0.05$, ** $P < 0.01$.

Figure 1.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GEN

Figure 2.

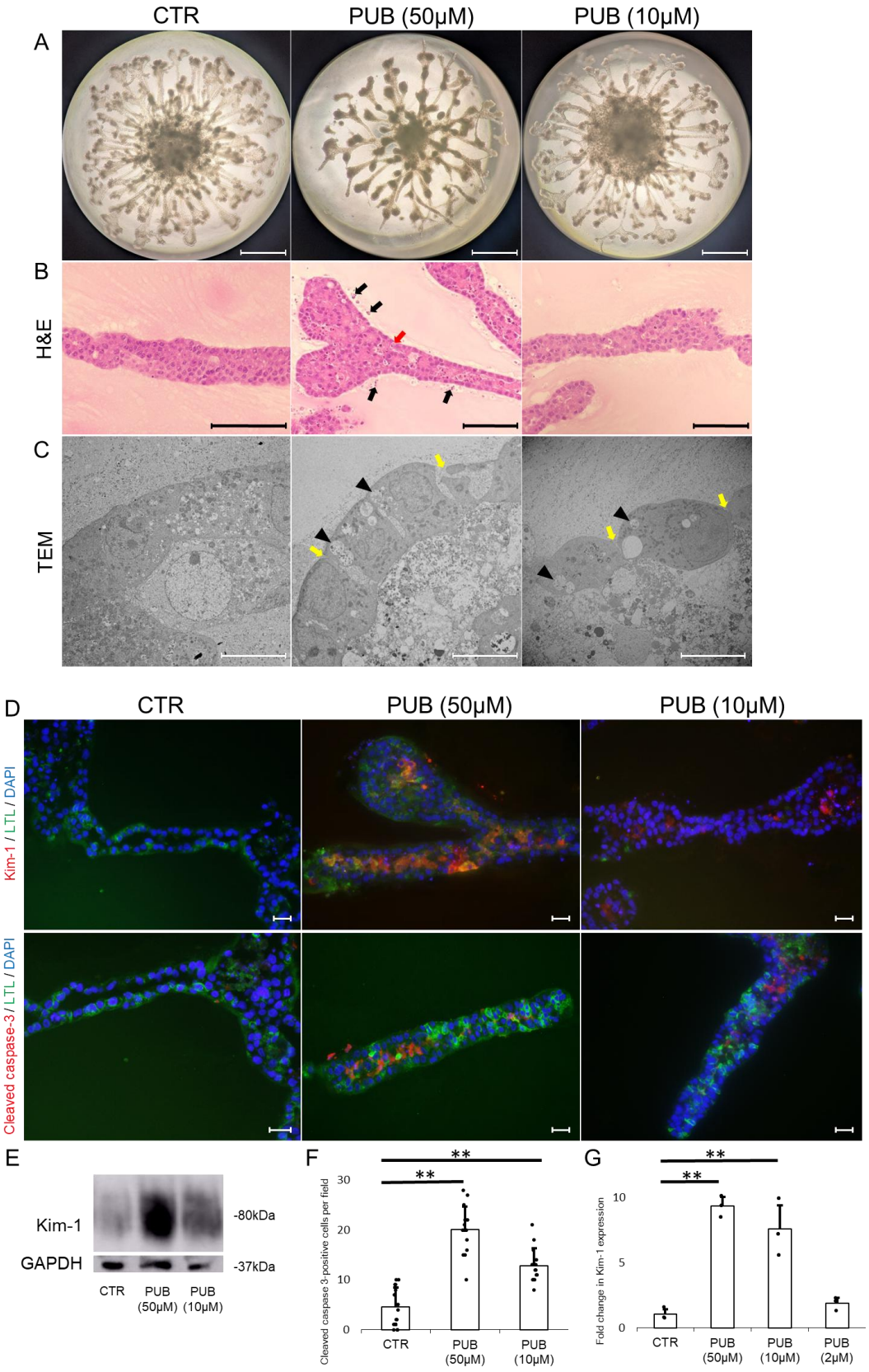
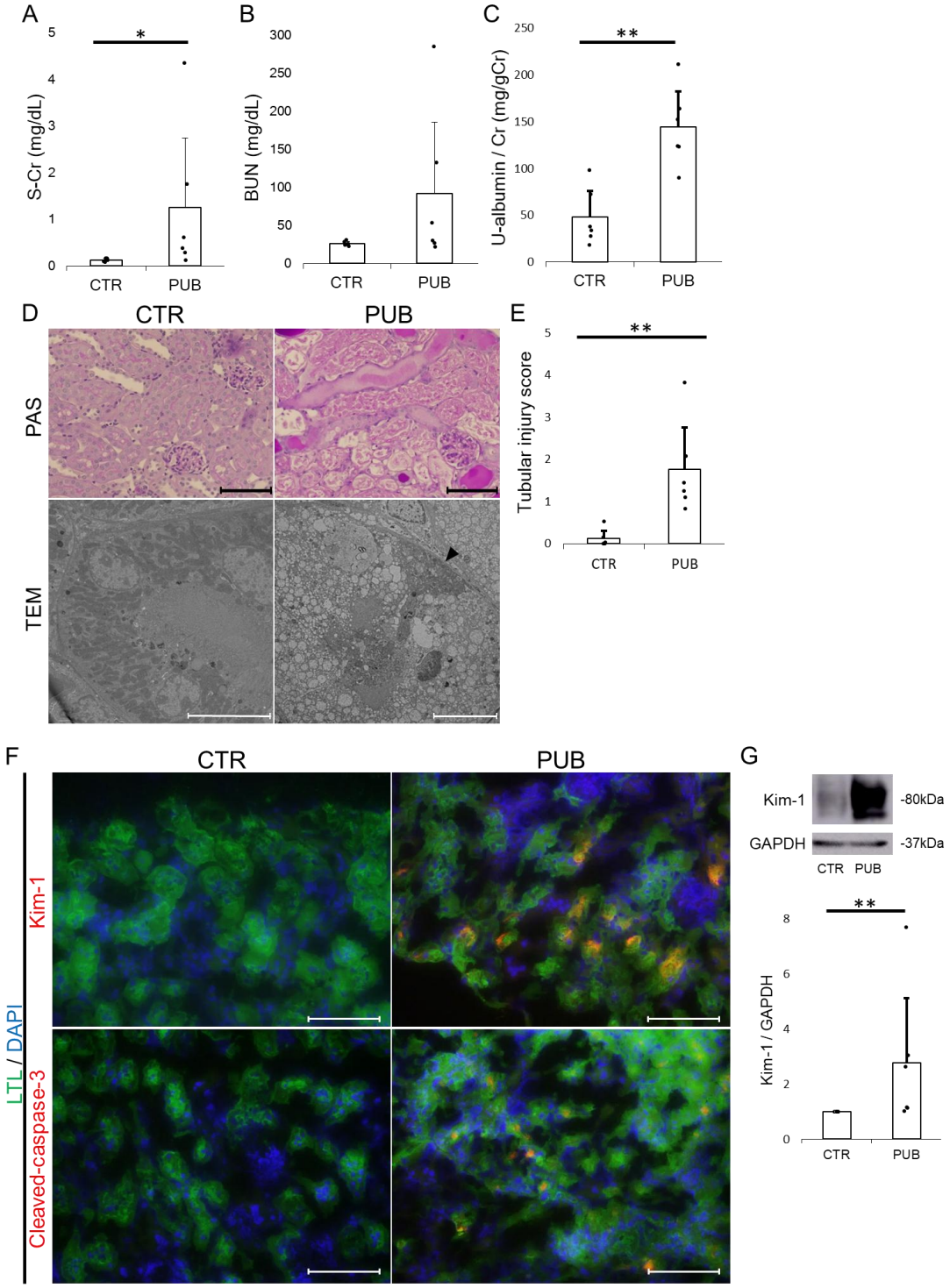

CTR
PUB (50μM)
PUB (10μM)
A
B
H&E
C
TEM
D
Kim-1 / LTL / DAPI
Cleaved caspase-3 / LTL / DAPI
E
Kim-1
GAPDH
-80kDa
-37kDa
CTR
PUB (50μM)
PUB (10μM)
F
Cleaved caspase 3-positive cells per field
30
20
10
0
**
**
CTR
PUB (50μM)
PUB (10μM)
G
Fold change in Kim-1 expression
10
5
0
**
**
CTR
PUB (50μM)
PUB (10μM)
PUB (2μM)

Figure 3.


A
S-Cr (mg/dL)
CTR
PUB
B
BUN (mg/dL)
C
U-albumin / Cr (mg/gCr)
D
PAS
TEM
E
Tubular injury score
F
Kim-1
Cleaved-caspase-3
LTL / DAPI
G
Kim-1
GAPDH
-80kDa
-37kDa
Kim-1 / GAPDH