Research Article

The Protective Effect of Fluorofenidone against Cyclosporine A-Induced Nephrotoxicity

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Keywords

Renal tubulointerstitial fibrosis · Apoptosis · Fluorofenidone · Cyclosporine A

Abstract

Background/Aims: Cyclosporine A (CsA) is an immunosuppressant drug that is used during organ transplants. However, its utility is limited by its nephrotoxic potential. This study aimed to investigate whether fluorofenidone (AKF-PD) could provide protection against CsA-induced nephrotoxicity. **Methods:** Eighty-five male Sprague-Dawley rats were divided into 5 groups: drug solvent, CsA, CsA with AKF-PD (250, 500 mg/kg/day), and CsA with pirfenidone (PFD, 250 mg/kg/day). Tubulointerstitial injury index, extracellular matrix (ECM) deposition, expression of type I and IV collagen, transforming growth factor (TGF)- β 1, platelet-derived growth factor (PDGF), Fas ligand (FASL), cleaved-caspase-3, cleaved-poly(ADP-ribose) polymerase (PARP)-1, and the number of transferase-mediated nick end-labeling (TUNEL)-positive renal tubule cells were determined. In addition, levels of TGF- β 1, FASL, cleaved-caspase-3, cleaved-PARP-1, and number of annexin V-positive cells were determined in rat proximal tubular epithelial cells (NRK-52E) treated with CsA (20 μ mol/L), AKF-PD (400 μ g/mL), PFD (400 μ g/mL), and GW788388 (5 μ mol/L). **Results:** AKF-PD (250, 500 mg/kg/day) significantly reduced tubulointerstitial injury, ECM deposition, expression of type I and IV collagen, TGF- β 1, PDGF, FASL, cleaved-caspase-3, cleaved-PARP-1, and number of TUNEL-positive renal tubule cells in the CsA-treated kidneys. In addition, AKF-PD (400 μ g/mL) significantly decreased TGF- β 1, FASL, cleaved-caspase-3, and PARP-1 expression in NRK-52E cells and further reduced the number of annexin V-positive cells. **Conclusion:** AKF-PD protect kidney from fibrosis and apoptosis in CsA-induced kidney injury.

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Introduction

Cyclosporine A (CsA) is an immunosuppressive drug commonly employed during organ transplant procedures, in part owing to its unique ability to suppress T cell responses without impairing myeloid cell activity [1]. Nevertheless, its administration is limited by its nephrotoxic potential [2]. Prolonged use of CsA can result in nephrotoxicity that is progressive, with permanent lesions forming that are associated with striped interstitial fibrosis and tubular atrophy [3].

Recent studies showed that apoptosis plays an important role in the pathogenesis of the chronic CsA lesion [4]. Proapoptotic signaling induced by CsA can be triggered through the extrinsic pathway, in which Fas ligand (FasL, CD95L) binds to and crosslinks the Fas receptor on the surface of target cells, activating an intracellular caspase cascades that can lead to tubular cell apoptosis [5–8].

Additionally, CsA directly upregulates transforming growth factor (TGF)- β 1 expression in tubular epithelial cells [9, 10]. TGF- β 1 is central to the fibrotic process. TGF- β 1 signaling promotes the synthesis of extracellular matrix (ECM) proteins, including fibronectin and collagens [11]. This ECM proteins production along with localized apoptosis is a major driver of renal tubular atrophy and tubulointerstitial fibrosis owing to the death of resident renal cells [12–14]. Previous studies have shown that TGF- β 1-induced apoptosis is driven by the upregulation of FasL in NRK-52E cells [15].

Fluorofenidone (AKF-PD; 1-[3-fluorophenyl]-5-methyl-2-[1H]-pyridone), a drug that has been demonstrated to possess multiorgan antifibrotic activity in the kidney [16–20], lung [21, 22], and liver [23, 24], offering the potential for renal protection. Moreover, previous study from our laboratory indicated that AKF-PD can inhibit Ang II-induced apoptosis of renal tubular cells through blockage of the Fas/FasL pathway [25]. Pirfenidone (PFD) has also been reported to attenuate renal fibrosis, preventing tubular cell apoptosis in a rat model CsA-induced chronic nephrotoxicity [26]. AKF-PD is structurally similar to PFD [27], and these 2 drugs further have similar antifibrotic activity [20].

In this article, we studied the effect of AKF-PD on renal structural and functional alterations induced by CsA and assessed whether the protective effect was associated with antifibrotic and antiapoptotic effects.

Materials and Methods

Antibodies and Reagents

Invitrogen (Carlsbad, CA, USA) was the source of all high glucose DMEM, fetal bovine serum (FBS), and penicillin/streptomycin. Antibodies against FasL were from Santa Cruz (Cambridge, MA, USA), while those against cleaved caspase-3 and PARP were from Cell Signaling Technology (Danvers, MA, USA). Collagen I and IV were purchased from Abcam (UK). Anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and CsA came from Sigma-Aldrich (St. Louis, MO, USA). TGF- β type I receptor inhibitor GW788388 was from Selleck (Shanghai, China). The Jackson Laboratory (Bar Harbor, ME, USA) was the source of horseradish peroxidase-conjugated secondary antibodies. Life Technologies (Carlsbad, CA, USA) was the source of Trizol, RevertAid First Strand cDNA Synthesis Kit, and Lipofectamine 2000. The SYBR green real-time polymerase chain reaction (PCR) kit came from Takara (Dalian, Liaoning, China). Roche (Mannheim, Germany) was the source of the transferase-mediated nick end-labeling (TUNEL) in Situ Cell Death Detection Kit, and the FITC Annexin V Apoptosis Detection Kit I was from BD Pharmingen (UK).

Animals

A total of 85 male Sprague-Dawley rats (215–500 g) from the Silaike Laboratory Animal (Shanghai, China) were housed under standard conditions, receiving a low-salt diet (0.05% sodium). CsA was dissolved in olive oil (Sinopharm chemical reagent, Shanghai, China) at 15 mg/mL. Randomization was used to divide

Table 1. The primers used for real-time PCR

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
Collagen I	TTGGGATGGAGGGAGTTTACACGA	TCAGGGGCGAAGGCAACAGT
Collagen IV	ATTCTTTGTGATGCACACCAG	AAGCTGTAAGCATTCGCGTAGTA
TGF-β1	CAACAATTCCTGGCG TTACCTT	AAGCCCTGTATTCCGTCTCCTT
PDGF	CTGAGCTGGACTTGAACATG	GCACTCGGCGATTACGG
β-Actin	GGAGATTACTGCCCTGGCTCCTA	GACTCATCGTACTCCTGCTTGCTG

PCR, polymerase chain reaction; TGF, transforming growth factor.

rats into 5 groups: drug solvent-treated males ($n = 17$); CsA-treated males (15 mg/kg/day, $n = 17$); CsA with AKF-PD treated males (250, 500 mg/kg/day, $n = 17$); and CsA with PFD treated males (250 mg/kg/day, $n = 17$). Synthesis of both AKF-PD and PFD was completed by the School of Pharmaceutical Sciences, Central South University. AKF-PD and PFD were both dissolved and diluted using 0.5% carboxymethyl cellulose sodium. Drug- and vehicle-treated groups received an oral gavage once daily beginning 18 days after injection. All rats were ultimately euthanized 36 days after the injection ($n = 85$). The left kidney of each rat was collected, and half was fixed using 4% paraformaldehyde prior to paraffin embedding. Sections (4 μ m thick) were cut for use in immunohistochemistry (IHC) and TUNEL staining procedures. The remaining kidney was snap frozen and was used for all quantitative PCR and Western blotting.

All animal protocols were compliant with NIH guidelines and met with the approval of the Institutional Animal Care and Use Committee of Xiangya School of Medicine, Central South University.

Renal Pathology and IHC

Paraffin sections were hematoxylin and eosin stained or were instead stained using Masson's trichrome, with hematoxylin and eosin sections being used to investigate renal tubulointerstitial injury in a semiquantitative manner [28]. Masson's stained sections were graded as previously described to assess collagen deposition [29]. The DAKO EnVision System (Dako Diagnostics, Zug, Switzerland) was used to complete all IHC studies. Initially, sections were stained with anticollagen I (1:500), or anticollagen IV (1:800). A blinded observer then assessed the degree of collagen staining via computerized morphometry with the Image Pro-Plus 6.0 software package (Media Cybernetics, Bethesda, MD, USA).

TUNEL Assay

Tubular epithelial cell apoptosis was assessed via terminal deoxynucleotidyl TUNEL staining. Tissue sections were processed according to the provided protocol, and TUNEL+ cells in 10 fields ($\times 100$) were independently counted by 2 blinded researchers.

Real-Time PCR

Trizol was employed to isolate total kidney RNA based on provided protocols, and 1 μ g of this RNA served as a template to synthesize cDNA with the ReverseAid first-strand cDNA synthesis kit. The SYBR Green PCR reagent kit was then used with an ABI Model 7900 Sequence Detector (Applied Biosystems, Foster City, CA, USA) to assess mRNA expression following normalization to β -actin, further calculation and statistical analysis were based on the comparative $2^{-\Delta\Delta CT}$ method. Primer sequences were shown in Table 1.

Cell Culture

NRK-52E cells from the American Type Culture Collection (Rockville, MD, USA) were grown under standard incubator conditions in DMEM containing 10% FBS, 100 U/mL penicillin, and 100 μ g/mL streptomycin. Cells were seeded in 12-well plates in complete medium for 24 h and were then switched to DMEM containing 2% FBS for 16 h. These synchronized NRK-52E cells were then separated into the following 5 groups: (1) control group: DMEM without CsA, AKF-PD, PFD or GW788388; (2) CsA (20 μ mol/L) group: CsA (20 μ mol/L)-treated group without AKF-PD, PFD or GW788388; (3) AKF-PD (400 μ g/mL) group: DMEM containing CsA (20 μ mol/L) and AKF-PD (400 μ g/mL); (4) PFD (400 μ g/mL) group: DMEM containing CsA (20 μ mol/L) and PFD (400 μ g/mL); (5) GW788388 (5 μ mol/L) group: DMEM containing CsA (20 μ mol/L) and GW788388 (5 μ mol/L). After 24 h, cells were harvested for further analysis.

Table 2. Changes in renal functional parameters

Group	n	BUN, mmol/L	PCr, $\mu\text{mol/L}$
Control	16	6.73 $\pm$ 0.98	49.50 $\pm$ 6.83
CsA	15	15.06 $\pm$ 3.21*	63.04 $\pm$ 9.10*
CsA+AKF-PD 250 mg $\times$ kg ⁻¹ $\times$ d ⁻¹	13	17.16 $\pm$ 5.16*	62.24 $\pm$ 11.96*
CsA+AKF-PD 500 mg $\times$ kg ⁻¹ $\times$ d ⁻¹	16	18.09 $\pm$ 11.33*	61.18 $\pm$ 6.65*
CsA+PFD 250 mg $\times$ kg ⁻¹ $\times$ d ⁻¹	15	19.35 $\pm$ 8.78*	73.89 $\pm$ 27.64*

All data are presented as means $\pm$ SD. * $p < 0.05$ versus control. BUN, blood urea nitrogen; CsA, cyclosporine A; PCr, plasma creatinine; AKF-PD, fluorofenidone; PFD, pirfenidone.

Assessment of Apoptosis

Annexin V-FITC/propidium iodide (PI) was employed to stain NRK-52E cells for apoptosis. Briefly, harvested cells were washed 2 times using cold PBS and incubated for 15 min in a binding buffer while protected from light with annexin V-FITC and PI. Staining was then assessed via flow cytometry (BD FACSCanto II UK).

Western Blot

Western blotting to assess levels of various proteins in renal or cell line samples were performed as previously described [16, 30]. Protein concentration was determined according to the manufacturer's protocol using the BCA Protein Assay Kit. Proteins were analyzed by 10 or 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (Millipore, USA). The membranes were incubated in a solution of Tris-buffered-saline (TBS) containing 5% (w/v) bovine serum albumin and 0.1% Tween-20 (TBST) for 1 h at room temperature before incubation overnight at 4 °C with primary antibodies against FasL (1:1,000), cleaved caspase-3 (1:1,000), cleaved PARP (1:1,000), or GAPDH (1:5,000). Membranes were washed 3 times with TBST. After washing, they were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. After washing 3 times with TBST, the membrane was then visualized using an enhanced chemiluminescent technique according to the manufacturer's instructions.

Statistical Analyses

All data are means $\pm$ SD, with SPSS version 18.0 (SPSS Inc., IL, USA) being used for all statistical testing. Between-group comparisons were via one-way ANOVA, with $p < 0.05$ being the statistical significance threshold.

Results

Functional Parameters

Changes in functional parameters after CsA treatment are shown in Table 2. In rats, CsA increased levels of both plasma creatinine and blood urea nitrogen relative to control group. AKF-PD (250, 500 mg/kg/day) or PFD (250 mg/kg/day) treatment had no effects on these parameters.

AKF-PD Attenuated Tubulointerstitial Injury and Fibrosis in CsA-Induced Nephropathy

Although vehicle-treated rats demonstrated normal kidney histology, CsA led to tubulointerstitial injury, as characterized by tubular dilatation and atrophy, infiltration by inflammatory cells into the interstitium, and localized fibrosis. AKF-PD treatment at either dose significantly decreased injury to tubulointerstitial structures as compared to CsA-treated rats. PFD (250 mg/kg/day) similarly reduced injury scores relative to rats treated with CsA alone (Fig. 1A). Kidney ECM deposition was assessed via Masson's trichrome staining,

Fig. 1. Representative microscopic findings. **A** AKF-PD (250, 500 mg/kg/day) and PFD (250 mg/kg/day) affected tubulointerstitial injury in the CSA-treated kidneys ($\times 200$). **B** AKF-PD (250, 500 mg/kg/day) and PFD (250 mg/kg/day) attenuated the deposition of ECM in the CSA-treated kidneys ($\times 100$). All data are presented as means $\pm$ SD, $n = 13$ –17; * $p < 0.05$ versus control, # $p < 0.05$ versus CsA, Δ $p < 0.05$ versus PFD 250 mg/kg/day. CsA, cyclosporine A; PFD, pirfenidone; AKF-PD, fluorofenidone.

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revealing that AKF-PD (250, 500 mg/kg/day) decreased the amount of collagen deposited in the interstitium relative to CsA-treated rats, as did PFD (250 mg/kg/day) treatment (Fig. 1B). Types I and IV collagen are the primary components of the ECM in the kidney that are associated with tubulointerstitial fibrosis. We therefore measured the levels of these collagens via IHC and real-time PCR. AKF-PD (250, 500 mg/kg/day) and PFD (250 mg/kg/day) resulted in significantly reduced levels of both type I and IV collagen (Fig. 2A–D).

AKF-PD-Inhibited PDGF and TGF- β 1 Expression in CsA-Induced Nephropathy

PDGF acts as a mitogenic compound for mesenchymal cells such as myofibroblasts, which are key fibrotic mediators, while TGF- β 1 further supports this process by stimulating additional collagen deposition from the dividing myofibroblasts [31, 32]. mRNA expression of TGF- β 1 and PDGF was elevated in CsA-treated rat kidneys, AKF-PD (250, 500 mg/kg/day), and PFD (250 mg/kg/day) attenuated those effects (Fig 3A, B).

AKF-PD Reduced Apoptosis in CsA-Induced Nephropathy

To determine the protective effects of AKF-PD on CsA-induced renal tubular apoptosis, we conducted TUNEL staining and found a greater number of TUNEL-positive apoptotic rat kidney cells following CsA treatment relative to vehicle controls. Both AKF-PD (250 and 500 mg/kg/day) and PFD (250 mg/kg/day) caused a significant decrease in apoptotic cell numbers in CsA-treated kidneys (Fig. 4A). CsA is also known to induce increases in proapo-

Fig. 3. AKF-PD (250, 500 mg/kg/day) and PFD (250 mg/kg/day) affected the relative mRNA expression of TGF- β 1 and PDGF in the CSA-treated kidneys. TGF- β 1 (**A**) and PDGF (**B**) mRNA expression in the kidneys. All data are presented as means $\pm$ SD, $n = 6$. * $p < 0.05$ versus control, # $p < 0.05$ versus CsA. CsA, cyclosporine A; PFD, pirfenidone; TGF, transforming growth factor; AKF-PD, fluorofenidone.

Fig. 2. AKF-PD (250, 500 mg/kg/day) and PFD (250 mg/kg/day) affected collagen I and collagen IV protein expression in the CSA-treated kidneys. Collagen I (**A**) and collagen IV (**B**) protein expression in the CSA-treated kidneys stained by IHC ($\times 200$). The histogram was the semiquantitative analysis result of collagen I and IV protein expression in the kidneys. Collagen I (**C**) and collagen IV (**D**) mRNA expression in the kidneys. All data are presented as means $\pm$ SD, $n = 6$. * $p < 0.05$ versus control, # $p < 0.05$ versus CsA, $\blacktriangle p < 0.05$ versus PFD 250 mg/kg/day. CsA, cyclosporine A; PFD, pirfenidone; AKF-PD, fluorofenidone.

Fig. 4. AKF-PD (250, 500 mg/kg/day) and PFD (250 mg/kg/day) affected tubular cell apoptosis in the CSA-treated kidneys. **A** Renal cortex was assayed for apoptosis via TUNEL staining ($\times 100$). Typical images and quantifications are shown. **B** Representative Western blot of FasL, cleaved-caspase-3, and cleaved-PARP-1 in rat kidney tissue. Quantitative analysis of FasL (**C**), cleaved-caspase-3 (**D**), and cleaved-PARP-1 (**E**) expression. All data represented as means $\pm$ SD, $n = 3$. * $p < 0.05$ versus control, # $p < 0.05$ versus CsA. CsA, cyclosporine A; PFD, pirfenidone; FasL, Fas ligand; AKF-PD, fluorofenidone.

ptotic markers such as FasL and the cleaved forms of caspase-3 and PARP-1, all of which were clearly downregulated in AKF-PD or PFD-treated kidneys (Fig. 4B–E).

AKF-PD Reduced mRNA Expression of TGF- β 1 and Apoptosis in CsA-Treated NRK-52E Cells

We next sought to determine how AKF-PD influences apoptosis in vitro. First, we found that the mRNA expression of TGF- β 1 was significantly increased in CsA-treated NRK-52E cells. Whereas, this change was restored by AKF-PD (400 μ g/mL) or PFD (400 μ g/mL) or TGF- β type I receptor inhibitor GW788388 (5 μ mol/L; Fig. 5F). Second, annexin V/PI staining was performed in order to measure the number of apoptotic cells by flow cytometry. As shown in Figure 5A, NRK-52E cells treated with CSA (20 μ mol/L) exhibited majority of cells in early apoptotic stage (49.87%) with 6.15% in the late apoptosis. Simultaneous treatment with AKF-PD (400 μ g/mL) or PFD (400 μ g/mL) or GW788388 (5 μ mol/L) and CsA was associated with a marked reduction in such apoptosis (Fig. 5A). Furthermore, western blotting was further performed to assess how treatment with these drugs affected CsA-induced caspase activation, PARP-1 cleavage, and upregulation of FasL. Results showed that FasL expression was increased, and that both caspase-3 and PARP-1 were activated during CsA treatment. AKF-PD (400 μ g/mL), PFD (400 μ g/mL), or GW788388 (5 μ mol/L) administration significantly reduced all 3 of these apoptotic indicators (Fig. 5B–E).

Discussion

Herein we demonstrated that AKF-PD remarkably attenuates kidney injury induced by CsA. AKF-PD administration prevented tubulointerstitial fibrosis and further led to a reduction in renal tubular epithelial cell apoptosis. Signaling and protein production associated with fibrotic activity and apoptosis were similarly reduced by these agents.

Chronic CsA nephrotoxicity is linked to striped interstitial fibrosis, tubular atrophy, and afferent arteriole hyalinosis [33]. In order to assess the antifibrotic activities of AKF-PD, we investigated ECM deposition and tubulointerstitial injury scores in rats that had been treated with CsA. We found that AKF-PD treatment reduced both of these pathogenic indices, and assessments of type I and IV collagen levels further confirmed the ability of this drug to protect against fibrogenesis in the context of chronic CsA administration.

During normal kidney development, apoptosis is important for maintaining normal tissue function [33, 34]. However, when apoptosis remains unchecked, it can lead to extensive and gradual kidney cell loss, culminating in renal tubular atrophy and fibrotic tubulointerstitial scarring [35]. The apoptotic death of tubular cells can ultimately create an ideal local micro-environment for the proliferation and activation of myofibroblasts and other fibrogenic cells owing to local transdifferentiation [36]. Significantly elevated apoptotic tubular cells were observed in renal biopsy specimens obtained from patients with CsA nephrotoxicity [37]. In this study, we found that the number of cells undergoing apoptosis increased in the tubular

Fig. 5. AKF-PD (400 μ g/mL), PFD (400 μ g/mL), and GW788388 (5 μ mol/L) suppressed apoptosis and mRNA expression of TGF- β 1 induced by CsA in NRK-52E cells. **A** Percentages of apoptotic cells determined by FACS analysis using annexin V-FITC and PI. **B** Representative Western blot of FasL, cleaved-caspase-3 and cleaved-PARP-1 in NRK-52E cells. Quantitative analysis of FasL (**C**), cleaved-caspase-3 (**D**), and cleaved-PARP-1 (**E**) expression in NRK-52E cells ($n = 3$). **F** The relative mRNA expression of TGF- β 1 in NRK-52E cells ($n = 6$). All data are presented as means $\pm$ SD. * $p < 0.05$ versus control, # $p < 0.05$ versus CsA. Δ $p < 0.05$ versus AKF-PD 400 μ g/mL. CsA, cyclosporine A; PFD, pirfenidone; TGF, transforming growth factor; FasL, Fas ligand; AKF-PD, fluorofenidone.

(For figure see next page.)

region upon CsA treatment. Consistent with the results of TUNEL staining of kidney samples, annexin V-PI staining of NRK-52E cells revealed an increase in apoptotic cells following CsA treatment. In both instances, AKF-PD significantly reduced the number of apoptotic cells. The cell surface receptor Fas and its ligand are key apoptotic mediators [38]. Caspase-3 activation similarly acts as a vital step in the process of determining whether or not a cell will progress to apoptosis, signaling via PARP-1. PARP-1 itself is degraded by caspase-3 into 2 cleavage fragments [39]. Our study suggests that the FasL, caspase-3, and PARP-1 are all implicated in CsA-induced apoptosis. AKF-PD significantly reduced the expression and/or activity of these apoptotic genes in both CsA-treated rats and NRK-52E cells.

TGF- β 1 is also believed to be a key player in the development of renal fibrosis, both promoting EMT and apoptosis in renal tubule cells and activating local myofibroblasts and epithelial cells that then produce additional ECM components and impair ECM degradation [40]. There is evidence to suggest that TGF- β 1 can enhance Fas-mediated apoptosis in primary cultured bronchiolar epithelial cells [41]. Similarly, TGF- β 1 also enhances Fas and FasL expression in NRK-52E cells [15]. In this study, we showed that AKF-PD could suppress the mRNA expression of TGF- β 1 induced by CsA treatment in NRK-52E cells and rats. Moreover, administration of TGF- β type I receptor inhibitor GW788388 significantly reduced the number of apoptotic cells and FasL/caspase-3 expression. As such, our observations revealed that TGF- β 1 is an important mediator of CSA-induced FasL/caspase-3 activation, and AKF-PD could suppress apoptosis induced by CsA partly through inhibition of TGF- β 1 signals.

PDGF is involved in mediating renal fibrosis, as it is normally expressed at low levels in healthy adult kidneys, but this expression is elevated in the context of fibrogenesis [42, 43]. In this study, we found that the level of PDGF was increased in kidneys during CsA treatment. Previous study indicated that CsA induced accumulation of PDGF mainly in the walls of renal arterioles [44]. Therefore, we prospectively inhibited PDGF expression in CsA-treated kidney possibly by affecting vasoconstriction of renal arterioles.

PFD is an antifibrotic molecule that was shown to prevent fibrosis effectively. It has been reported to attenuate renal fibrosis [26], preventing tubular cell apoptosis in a rat model CsA-induced chronic nephrotoxicity. AKF-PD has the similar chemical structure with PFD, but the difference of its molecular structure from that of PFD is that one hydrogen atom at the meta-position of the benzene ring in PFD is replaced by a fluorine atom in AKF-PD. Since fluorine features the highest electronegativity among all elements and the carbon – fluorine bond has considerable stability, while the toxicity of AKF-PD was poorer [27]. Our finding indicated that these 2 drugs have similar therapeutic effects against CsA-induced nephrotoxicity by decreasing the expression of proapoptotic proteins and ECM deposition.

In summary, our findings show that AKF-PD attenuates experimental CsA-induced kidney injuries through antifibrotic and antiapoptotic effects. AKF-PD therefore may represent an alternative or additional therapeutic approach for CsA-induced nephrotoxicity.

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Statement of Ethics

All animal protocols were compliant with NIH guidelines and met with the approval of the Institutional Animal Care and Use Committee of Xiangya Hospital, Central South University.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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