Biochemical and Biophysical Research Communications 444 (2014) 121–127

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Renoprotective effect of paricalcitol via a modulation of the TLR4-NF-κB pathway in ischemia/reperfusion-induced acute kidney injury

Jae-Won Lee [1], Sun Chul Kim [1], Yoon Sook Ko, Hee Young Lee, Eunjung Cho, Myung-Gyu Kim, Sang-Kyung Jo, Won Yong Cho *, Hyoung Kyu Kim

Division of Nephrology, Department of Internal Medicine, Korea University Medical College, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 24 December 2013
Available online 14 January 2014

Keywords:
Acute kidney injury
NF-κB
Paricalcitol
Toll-like receptor 4

ABSTRACT

Background: The pathophysiology of ischemic acute kidney injury (AKI) is thought to include a complex interplay between vascular endothelial cell dysfunction, inflammation, and tubular cell damage. Several lines of evidence suggest a potential anti-inflammatory effect of vitamin D in various kidney injury models. In this study, we investigated the effect of paricalcitol, a synthetic vitamin D analog, on renal inflammation in a mouse model of ischemia/reperfusion (I/R) induced acute kidney injury (AKI).
Methods: Paricalcitol was administered via intraperitoneal (IP) injection at 24 h before ischemia, and then I/R was performed through bilateral clamping of the renal pedicles. Twenty-four hours after I/R, mice were sacrificed for the evaluation of injury and inflammation. Additionally, an *in vitro* experiment using HK-2 cells was also performed to examine the direct effect of paricalcitol on tubular cells.
Results: Pre-treatment with paricalcitol attenuated functional deterioration and histological damage in I/R induced AKI, and significantly decreased tissue neutrophil and macrophage infiltration and the levels of chemokines, the pro-inflammatory cytokine interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1). It also decreased IR-induced upregulation of Toll-like receptor 4 (TLR4), and nuclear translocation of p65 subunit of NF-κB. Results from the *in vitro* study showed pre-treatment with paricalcitol suppressed the TNF-α-induced depletion of cytosolic IκB in HK-2 cells.
Conclusion: These results demonstrate that pre-treatment with paricalcitol has a renoprotective effect in ischemic AKI, possibly by suppressing TLR4-NF-κB mediated inflammation.

1. Introduction

Ischemic acute kidney injury (AKI) is a significant clinical problem that results in a poor outcome in hospitalized patients. It is also associated with delayed and poor long-term graft function in kidney transplantation [1–3]. Renal ischemia/reperfusion (I/R) is one of the major causes of AKI, and there have been many advances in the understanding of the mechanisms mediating the I/R injury or repair processes. However, the clinical management of ischemic AKI remains largely supportive.

The pathophysiology of renal IR injury is thought to involve a complex interplay between vascular endothelial cell dysfunction, inflammation, and tubular cell damage. Inflammation in particular is a major component in the pathogenesis of renal IR injury, causing tissue damage by releasing several mediators such as pro-inflammatory cytokines, proteases, and eicosanoids [4]. Tubular cell apoptosis also plays an important role in renal dysfunction in ischemic AKI [5].

Paricalcitol (19-nor-1,25-dihydroxyvitamin D2) is an active, non-hypercalcemic vitamin D analog that shows similar biological activity, but has fewer adverse events and good tolerance [6]. Several recent observations have suggested that the effects of vitamin D analog are not limited to mineral bone metabolism, but also extend to cellular proliferation and immune modulation, suggesting the presence of pleiotropic action [7–9].

Active vitamin D and its analogs attenuate glomerular and tubular interstitial fibrosis, [10] and demonstrate therapeutic potential in various kidney injury models, including drug-induced AKI such as cyclosporine, gentamicin [11,12]; glomerulonephritis, and diabetic nephropathy [13,14]. Moreover, vitamin D has been reported to protect tissue from ischemic injury such as myocardial ischemia, cerebral ischemia, and muscle injury by peripheral arterial occlusion [15–17]. Although its ability to modulate inflammation has been suggested to be one of the potential mechanisms,

* Corresponding author. Address: Department of Internal Medicine, Korea University Medical College, 5Ka, Anam-Dong, Sungbuk-Gu, Seoul 136-705, Republic of Korea.

E-mail addresses: maestro97@hanmail.net (J.-W. Lee), linefe99@hanmail.net (S.C. Kim), rainboweyes@hanmail.net (Y.S. Ko), cell1023@hanmail.net (H.Y. Lee), icdej@naver.com (E. Cho), gyu219@hanmail.net (M.-G. Kim), sang-kyung@korea.ac.kr (S.-K. Jo), wonyong@korea.ac.kr (W.Y. Cho), hyoung@korea.ac.kr (H.K. Kim).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2014 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.bbrc.2014.01.005

little is known about the precise mechanism of beneficial effect of paricalcitol in ischemic injury.

In this study, we investigated the effect of paricalcitol on kidney injury and inflammation, focusing on the Toll-like receptor 4 (TLR4) – the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway in I/R induced AKI.

2. Materials and methods

2.1. Animal experiment

Six-to-eight week old, male C57BL/6 mice (weight, 20 ~ 22 g) were purchased from Orient Bio (Seongnam, Republic of Korea) and had free access to food and water before manipulation. Animal care was governed by the Animal Care Committee of Korea University for the care and the use of laboratory animals in research. Mice were either treated with 25 μg/kg of paricalcitol (Abbott, Italy), or the same volume of control vehicle administered through IP injection at 24 h before ischemia. Mice were subjected to bilateral renal pedicle clamping for 28 min, and sham operations were performed in a similar surgical procedure except for the clamping of renal pedicles. Twenty-four hours after IR, mice were sacrificed. Blood was collected by intracardiac puncture and kidneys were processed for molecular and histological examinations.

2.2. Biochemical analysis

Four hundred microliters of blood was collected at 24 h after I/R, and blood urea nitrogen (BUN), serum creatinine, and calcium were measured using a Hitachi 747 automatic analyzer.

2.3. Histological examination

The 10% formalin-fixed, paraffin-embedded, 4-μm thickness kidney sections were stained with periodic acid-Schiff reagent (PAS [Sigma Chemical Co, Perth, Australia]). Histological changes in the outer stripe of the outer medulla were assessed by quantitative measurements of tissue damage as described by Miyaji et al. [18]. Tubular damage was defined as tubular epithelial swelling, loss of brush border, vacuolar degeneration, necrotic tubules, cast formation, and desquamation. The degree of kidney damage was estimated at a 200× magnification using 10 randomly selected fields for each kidney by the following criteria: 0, normal; 1, areas of damage <25% of tubules; 2, damage involving 25–50% of tubules; 3, damage involving 50–75% of tubules; and 4, damage involving 75–100% of tubules.

Tubular cell apoptosis was examined by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining. For the immunohistochemical detection of monocytes, macrophages, or neutrophils, kidney tissues were stained with a monoclonal antibody against F4/80 (AbD Serotec, Kidlington, UK) or Ly6G (eBioscience, San Diego, CA, USA). Ten high power fields (HPF) using a 200× magnification of the outer stripe of the outer medulla were captured, and the mean numbers of TUNEL, F4/80- or Ly6G-positive cells per HPF were compared. Immunohistochemical analysis of TLR4 (Santa Cruz Biotechnology, Santa Cruz, CA, USA expression) was also performed, and quantitative measurement of TLR4-positive cells was done in the same manner.

2.4. Quantification of cytokines and chemokines by a cytometric bead array (CBA)

Quantification of cytokines and chemokines in kidney tissues was done using a cytometric bead array (CBA). A mouse inflammation kit (BD Bioscience, San Diego, CA, USA), was used according to the manufacturer's instructions to simultaneously detect mouse interleukin (IL)-12p70, tumor necrosis factor alpha (TNF)-α, interferon (IFN)-γ, monocyte chemotactic protein (MCP)-1, IL-10, and IL-6. Briefly, a mixture of 6 capture bead populations (50 μl) with distinct fluorescence intensities (detected in FL3) coated with antibodies specific for the above cytokines and chemokines were mixed with each sample from kidney homogenates. Standard dilutions and test samples were added to the appropriate sample tubes (50 μl/tube). Additionally, phycoerythrin (PE)-conjugated detection antibodies (detected in FL-2; 50 μl) were added to form sandwich complexes. After 2 h of incubation at room temperature, the samples were washed with 1 ml of wash buffer and centrifuged (200*g* for 5 min). Three hundred microliters of wash buffer was added to each assay tube before acquisition in a FACScan flow cytometer (FACSCalibur™; BD Biosciences), and the sample results were analyzed using CBA software (BD Biosciences). The concentration of each cytokine in cell supernatants was determined by interpolation from the corresponding standard curve and normalized according to protein concentrations. The range of detection was 20–5000 pg/ml for each cytokine.

2.5. Cell culture and cytokine treatment

To examine the direct effect of paricalcitol on the NF-κB pathway in tubular cells, an *in vitro* experiment using human proximal tubular epithelial cell line, HK-2 cells (American Type Culture Collection [ATCC], Manassas, VA, USA), was performed. Briefly, cells were passaged every 3–4 days in 100-mm dishes using Dulbecco's modified Eagle's medium-F12 (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Life Technologies, Gaithersburg, MD, USA), 100 U/ml penicillin, and 100 mg/ml streptomycin (Sigma–Aldrich). These cells were incubated in a humidified atmosphere of 5% CO_2, 95% air at 37.1 °C for 24 h and sub-cultured at 70–80% confluence. For experimental use, HK-2 cells were plated onto 60 mm dishes in medium containing 10% fetal bovine serum for 24 h. Cells were then switched to Dulbecco's modified Eagle's medium-F12 with 2% fetal bovine serum for 16 h. These cells were then treated with TNF-α (3 or 30 ng/ml) in the presence or absence of paricalcitol (0.2 ng/ml) for 4 h. Another set of HK-2 cells was treated with paricalcitol alone (0.2 ng/ml) and control cells received buffer only, instead of TNF-α or paricalcitol. The cells were harvested at the end of the treatment for further analysis.

2.6. Western blot analysis

With the preparation of whole-cell lysates and kidney tissue homogenates, Western blot analyses of protein expression were carried out by using routine procedures, as described previously. The primary antibodies were obtained from following sources: anti-p65 NF-κB, and anti-IκBα (Cell Signaling Technology, Danvers, MA, USA). Anti ß-actin (Santa Cruz Biotechnology, CA, USA) or anti lamin B (Cell Signaling Technology) was used for loading controls.

Table 1
Attenuation of renal functional impairment after IR by paricalcitol pre-treatment.

	Sham	IR	Paricalcitol + IR
n	18	26	26
BUN (mg/dL)	18 ± 1.3	105.7 ± 11.5 [a]	35.0 ± 4.9 [b]
SCr (mg/dL)	0.23 ± 0.01	1.29 ± 0.10 [a]	0.31 ± 0.03[b]
SCa (mg/dL)	10.05 ± 1.35	9.25 ± 0.40 [c]	9.62 ± 0.29 [d]

Abbreviations: BUN; blood urea nitrogen, SCr; serum creatinine, SCa; serum calcium, IR; ischemia–reperfusion.
Values are expressed as the mean ± s.e.m.
[a] $p < 0.05$ compared with sham.
[b] $p < 0.05$ compared with I/R mice.
[c] $p = 0.338$ compared with sham.
[d] $p = 0.726$ compared with I/R mice.

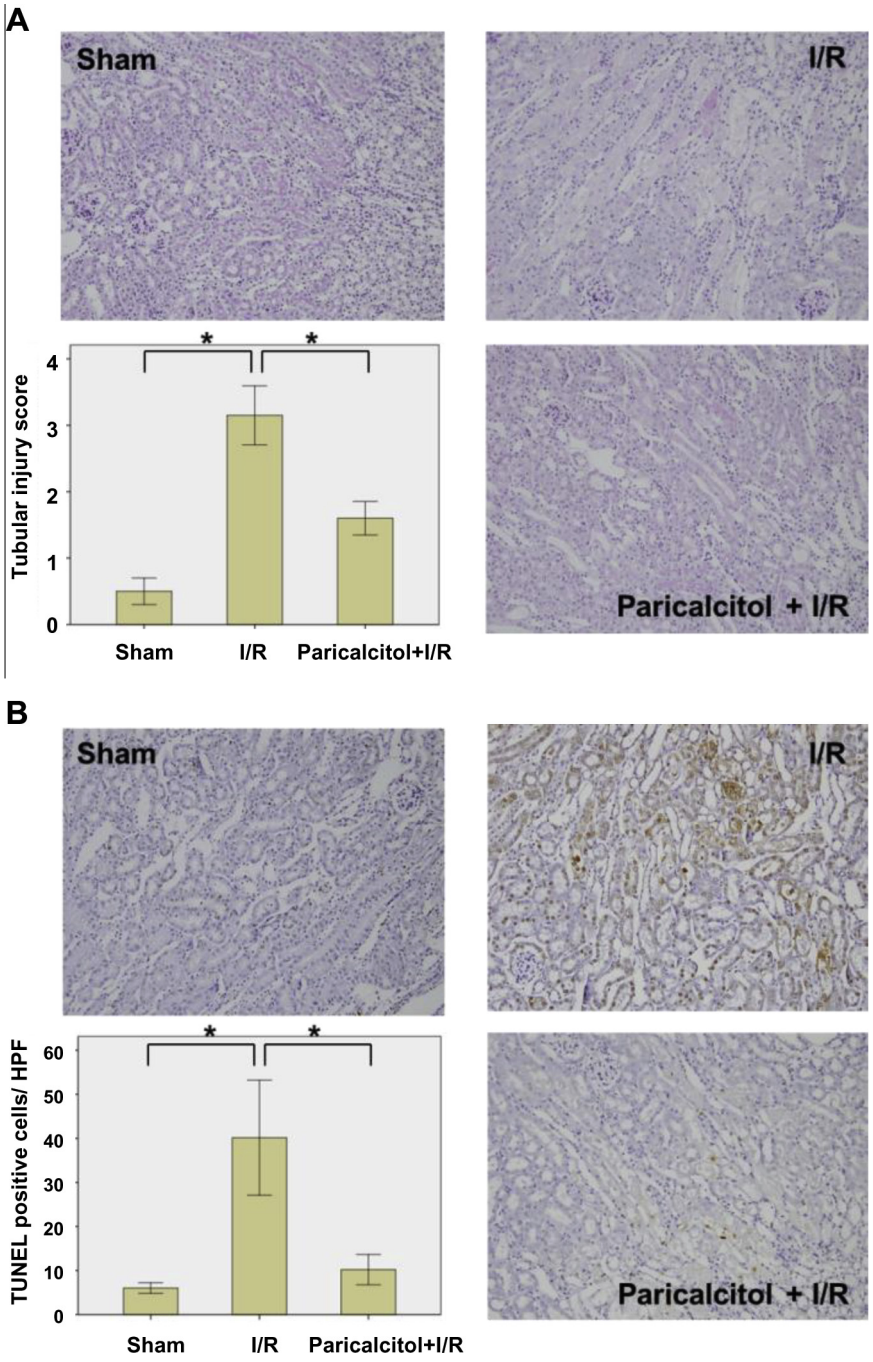

Fig. 1. Effect of paricalcitol on tubular cell damage and apoptosis. (A) Tubular epithelial swelling, loss of brush border, vacuolar degeneration, necrotic tubules, cast formation, and desquamation were noticed in mice in response to I/R injury. Paricalcitol ameliorated these changes (PAS stain, 100×). (B) The TUNEL assay revealed increased apoptosis in response to I/R injury, whereas paricalcitol pre-treatment significantly reduced the number of TUNEL-positive cells (TUNEL stain, 100×). * $p < 0.05$ compared with sham or I/R.

2.7. Statistical analysis

All data were presented as the mean ± SE and were analyzed by a Kruskal–Wallis's test. A p value of less than 0.05 was considered statistically significant.

3. Results

3.1. Pre-treatment of paricalcitol attenuated kidney injury

Pre-treatment with paricalcitol attenuated the elevation of BUN (105.7 ± 11.5 mg/dL versus 35.0 ± 4.9 mg/dL, $p < 0.05$), and creatinine (1.29 ± 0.01 mg/dL versus 0.31 ± 0.03 mg/dL, $p < 0.05$) levels at Day 1 after I/R injury, but did not affect serum calcium levels (Table 1). Histological kidney injury such as epithelial swelling, loss of brush border, vacuolar degeneration, necrotic tubules, cast formation, and desquamation were all observed in vehicle-treated mice, but pre-treatment with paricalcitol attenuated histologic kidney injury (Fig. 1A). The number of TUNEL positive tubular cell apoptosis was also decreased by paricalcitol pre-treatment (Fig. 1B).

3.2. Pre-treatment of paricalcitol attenuated renal inflammation

To determine the effect of paricalcitol on renal inflammation, the infiltration of Ly6G-positive neutrophils and F4/80-positive macrophages were analyzed. Increased neutrophil and macrophage

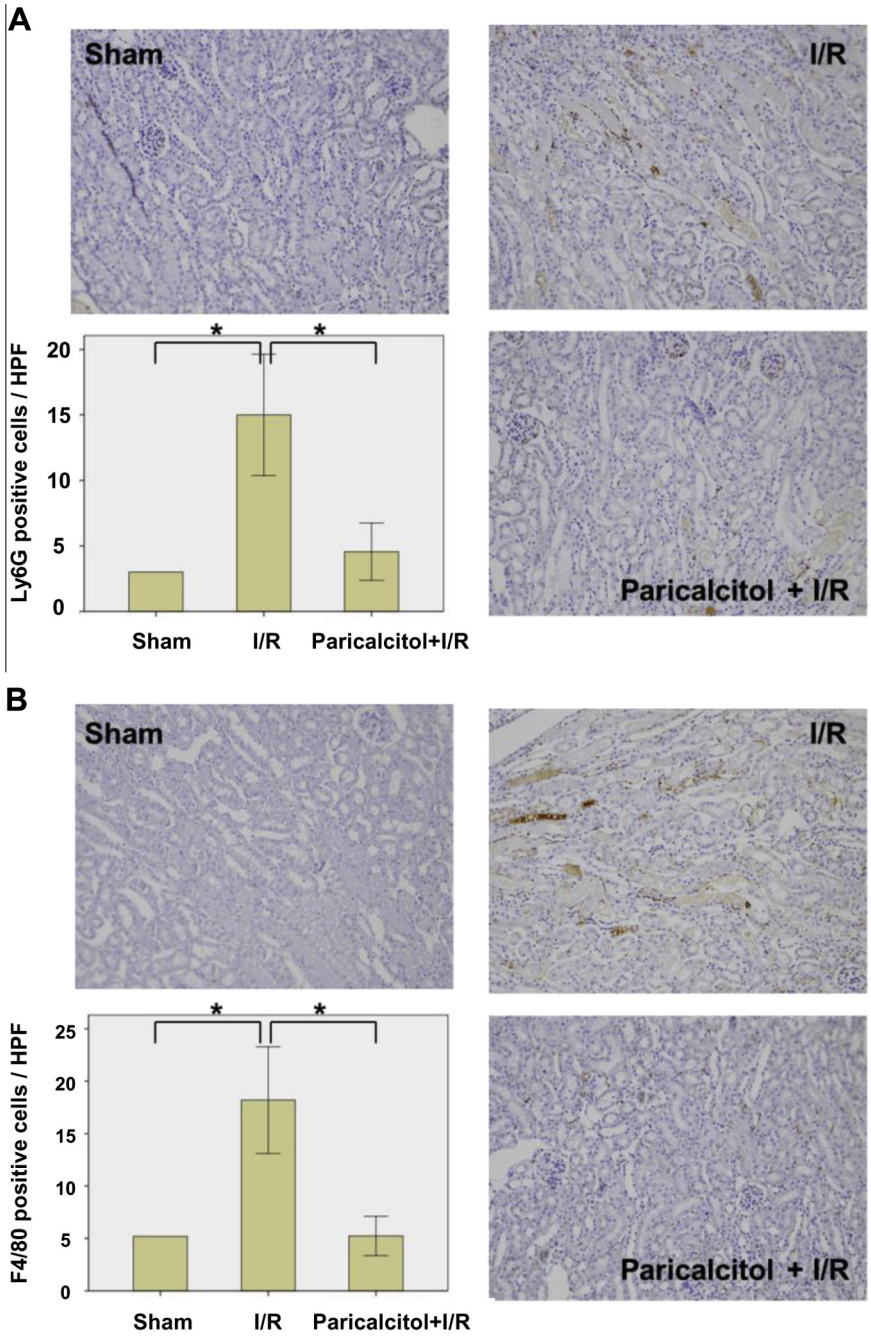

Fig. 2. Effects of paricalcitol on inflammatory cell infiltration. The infiltration of Ly6G-positive cells (A) or F4/80-positive cells (B) increased significantly in I/R mice, which was ameliorated by paricalcitol pre-treatment (100×). * $p < 0.05$ compared with sham or I/R.

infiltration at 24 h after reperfusion were decreased significantly by paricalcitol pre-treatment (Fig. 2). In addition, we also observed that interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) levels, which were markedly increased at 24 h after reperfusion, were significantly decreased in paricalcitol pre-treated I/R mice (Fig. 3).

3.3. Pre-treatment of paricalcitol suppressed TLR4 and NF-κB activation in kidney

To further examine whether pre-treatment with paricalcitol modulates TLR4 signaling, an important inflammatory pathway mediating ischemic AKI, we first analyzed the expression of TLR4 in tubular cells. We found that I/R increased TLR4 expression in tubular epithelial cells, but pre-treatment with paricalcitol markedly attenuated TLR4 expression (Fig. 4A). In the analysis of NF-κB, the downstream molecule of TLR4 pathway, an increase in p65 subunit of NF-κB was observed in the kidney of I/R mice, but this was also suppressed by paricalcitol pre-treatment (Fig. 4B).

3.4. Paricalcitol prevented the TNF-α-induced decrease in cytosolic IκB in cultured tubular cells

Results from an *in vitro* experiment designed to show the direct effect of paricalcitol treatment on tubular cells showed that the

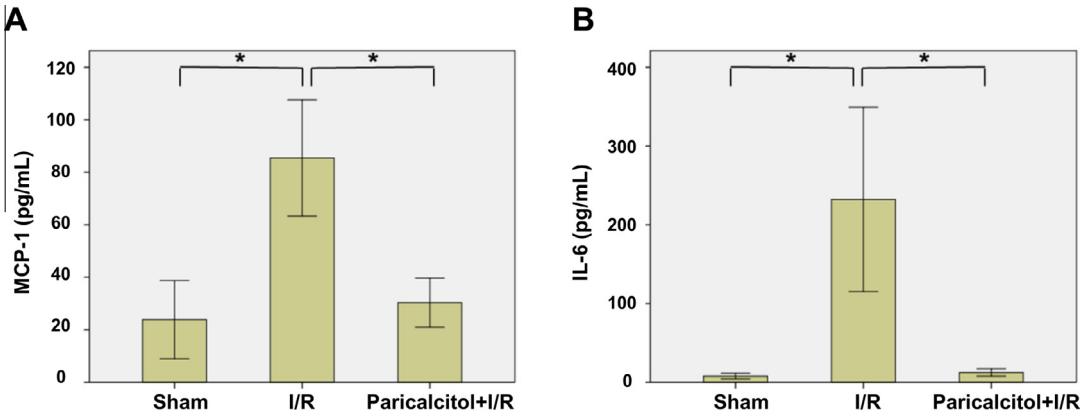

Fig. 3. Effects of paricalcitol on inflammatory chemokines and cytokines. Inflammatory chemokine, MCP-1 (A), and cytokine, IL-6 (B), were significantly elevated in I/R mice compared to those in control. Paricalcitol pre-treatment suppressed the I/R-induced overexpression of these inflammatory cells. * $p < 0.05$ compared with sham or I/R.

expression of cytoplasmic IκB was significantly decreased in HK-2 cells by TNF-α in dose-dependent manner, and decreased cytoplasmic IκB was partially prevented by paricalcitol treatment (Fig. 4C).

4. Discussion

This study demonstrated that pre-treatment with paricalcitol had a renoprotective effect by attenuating inflammation in I/R induced AKI in mice. To further elucidate possible mechanisms mediating the anti-inflammatory effect of paricalcitol, we examined the expression of TLR4 and NF-κB in a I/R induced AKI mice model, and found that the tubular expression of TLR4 and NF-κB were increased by I/R injury and suppressed by pre-treatment with paricalcitol. The *in vitro* study showed the expression of cytoplasmic IκB were decreased in HK-2 cells by TNF-α, and this finding was partially reversed by paricalcitol. These results suggest that paricalcitol improved renal inflammation and damage by blocking the activation of TLR4-NF-κB pathway in ischemic AKI.

TLR4 is a trans-membrane protein that plays a central role in immune response, including NF-κB regulation [19]. I/R injury produces endogenous ligands referred to damage-associated molecular pattern molecules (DAMPs) and leads to the expression of TLR4 in renal tubular cells. The activation of tubular TLR4 by DAMPs propagates the initial injury through development of subsequent inflammatory processes, including the production of proinflammatory cytokines and chemokines and infiltration of immune cells in ischemic AKI [20,21]. This subsequent inflammatory process via TLR4 is mediated by increased expression of nuclear NF-κB through a MyD88-dependent pathway [22,23].

NF-κB is a key transcription factor that regulates the expression of a number of genes, including those encoding chemokines, adhesion molecules, and cytokines [24,25] such as TNF-α and MCP-1, which have κB-binding motifs in their promoter regions. The NF-κB signaling ultimately initiates the inflammatory cascade and leukocyte recruitment. These results support that TLR4 stimulation and subsequent activation of the NF-κB pathway could be a potential therapeutic target in ischemic AKI.

Several reports have demonstrated that vitamin D reduces the expression of TLR4 in human monocytes [26], myometrial cells [27], human colon cancer cell lines (Caco-2, HCT116 and HT-29) [28], rat aortic injury model [29], and a rat nonalcoholic steatohepatitis model [30]. However, the role of paricalcitol on TLR4 expression in ischemic kidney has not been determined yet. This study is the first to demonstrate that pre-treatment with paricalcitol may reduce the expression of TLR4 in renal tubular cells and subsequent inflammatory processes following I/R injury.

Recently, a study showed that COX-2 and PGE2 expression were increased by paricalcitol which may contribute to renoprotection and reduced inflammation in I/R induced AKI [31]. However, there are some conflicting results about the role of COX-2 and PGE2 expression in ischemic AKI studies [32]. Therefore, we focused on the more up-stream components of these inflammatory pathways and found that paricalcitol treatment modulates the TLR4-NF-κB pathway in the I/R induced AKI model [33,34]. Although we did not examine the level of COX-2 and PGE2 in this study, the changes in other cytokine and chemokine levels were investigated, and significant reductions of IL-6 and MCP-1 by paricalcitol treatment were observed. In the ischemic kidney, MCP-1 plays an important role in leukocyte recruitment, further compromising the outer medullary reflow and facilitating tubular cell damage [35,36]. IL-6 is also an important pro-inflammatory cytokine in renal I/R injury. Previously, Kielar et al., demonstrated an important role for IL-6, showing that IL-$6^{-/-}$ mice are protected against ischemic injury [37]. In addition, a study using human living-donor allograft revealed that early renal I/R injury is dominated by a local release of IL-6 [38]. Therefore, a reduction of IL-6 and MCP-1 via the modulation of TLR4-NF-κB pathway might be considered to mediate the renoprotective effect of paricalcitol pre-treatment in renal I/R injury.

The renoprotective effect of vitamin D through its impact on inhibiting NF-κB activation was previously demonstrated in an obstructive nephropathy model [39]. In addition, *in vitro* studies using HK-2 cells showed that paricalcitol attenuated NF-κB activation induced by cyclosporine or gentamicin [11,12]. Similarly, our study showed that the increased expression of NK-κB in I/R kidney was decreased by paricalcitol treatment. Additionally, our *in vitro* findings showed that the decreased expression of IκB in TNF-α-treated HK-2 cells was partially reversed by paricalcitol. However, unlike a previous study [39] that showed that paricalcitol treatment sequestered NF-κB signaling by interfering with the binding of p65 to the RANTES promoter in nucleus without any change upon IκB degradation, in our study, paricalcitol treatment affected IκB degradation. The discrepancy in these findings could have resulted from differences in experimental settings such as mouse strain, different doses or administration route of paricalcitol, and a different time point for the assessment of inflammation.

In this study, the dose of paricalcitol (25 μg/kg, IP) was higher than that of previous studies. In a obstructive nephropathy model using CD-1 mice, 0.1 or 0.3 μg/kg of paricalcitol was administered by daily subcutaneous injection for 7 or 14 days [39]. In drug-induced AKI models using either cyclosporine or gentamicin and Sprague-Dawley rats, 0.2 or 0.3 μg/kg of paricalcitol was injected

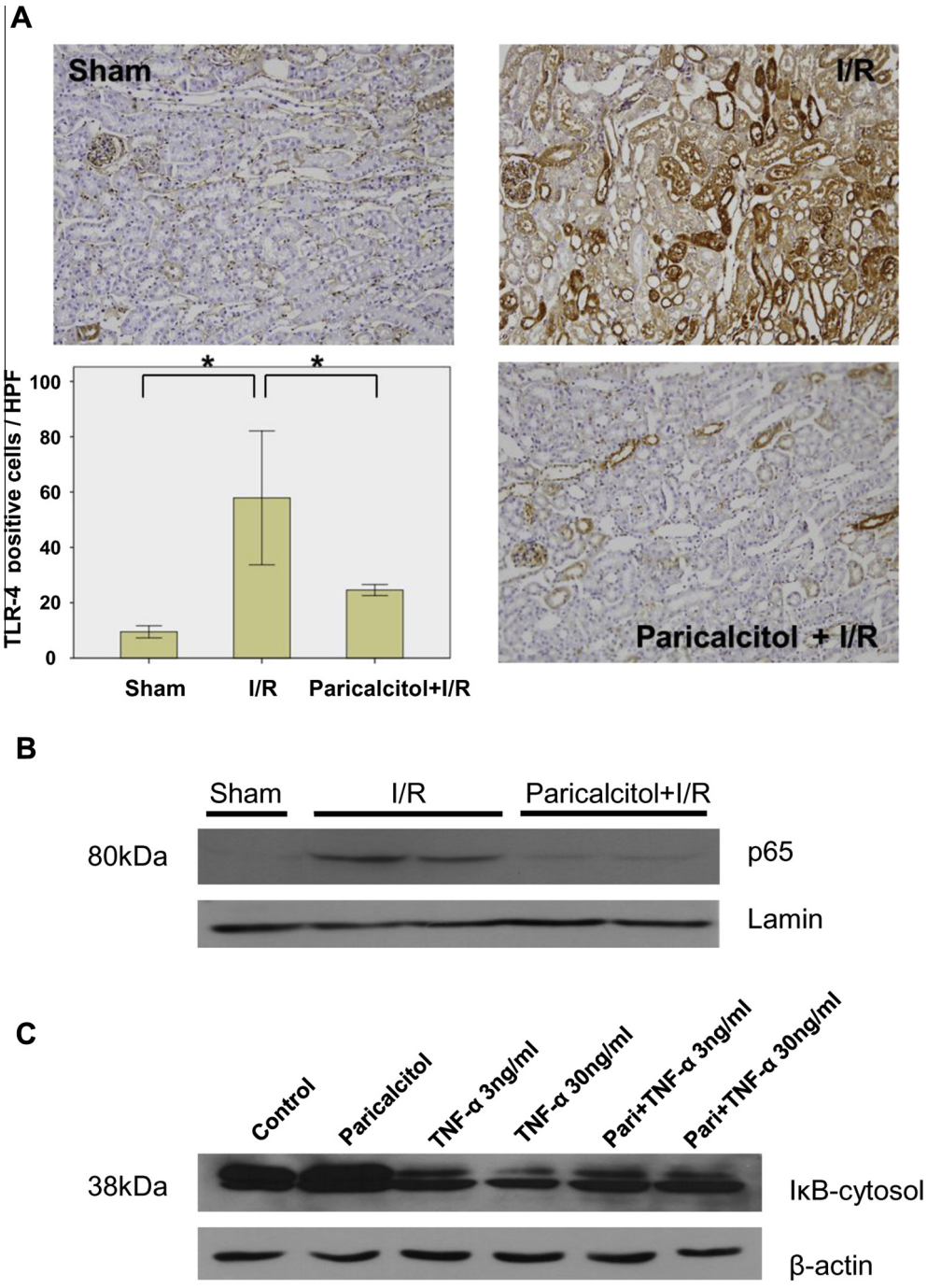

Fig. 4. Effects of paricalcitol on TLR4-NF-κB pathway. (A) The increased expression of TLR4 in tubular cells was noticed in response to I/R injury, whereas paricalcitol pre-treatment significantly reduced TLR4 expression (100×). (B) The expression of p65 NF-κB increased after I/R injury, and this was attenuated by paricalcitol pre-treatment. (C) The expression of cytosolic IκB in HK-2 cells was decreased significantly by TNF-α in proportion to TNF-α concentration. The depletion of cytosolic IκB was suppressed by paricalcitol. * $p < 0.05$ compared with sham or I/R.

by subcutaneously for 14 or 28 days [11,12]. In our study, paricalcitol was administered only once, and no toxic effects (such as hypercalcemia) were noted.

In conclusion, pre-treatment with paricalcitol was renoprotective in ischemic AKI and was associated with the suppression of TLR4-NF-κB signaling. Subsequently, decreased inflammatory molecules and leukocyte recruitment were observed, suggesting that the modulation of TLR4-NF-κB signaling may play a role in the renoprotective effect of paricalcitol pre-treatment. In various clinical settings, paricalcitol has been widely used with fewer adverse events and good tolerance; therefore, our results indicate that paricalcitol might be a promising agent for clinical application to ischemic AKI.

References

[1] R.A. Star, Treatment of acute renal failure, Kidney Int. 54 (1998) 1817–1831.
[2] J.V. Bonventre, J.M. Weinberg, Recent advances in the pathophysiology of ischemic acute renal failure, J. Am. Soc. Nephrol. 14 (2003) 2199–2210.
[3] M.A. Venkatachalam, K.A. Griffin, R. Lan, H. Geng, P. Saikumar, A.K. Bidani, Acute kidney injury: a springboard for progression in chronic kidney disease, Am. J. Physiol. Renal Physiol. 298 (2010) F1078–F1094.
[4] M.D. Okusa, The inflammatory cascade in acute ischemic renal failure, Nephron 90 (2002) 133–138.

[5] R. Bonegio, W. Lieberthal, Role of apoptosis in the pathogenesis of acute renal failure, Curr. Opin. Nephrol. Hypertens. 11 (2002) 301–308.
[6] T.B. Drueke, Which vitamin D derivative to prescribe for renal patients, Curr. Opin. Nephrol. Hypertens. 14 (2005) 343–349.
[7] T. Weinreich, J. Merke, M. Schonermark, H. Reichel, M. Diebold, G.M. Hansch, E. Ritz, Actions of 1,25-dihydroxyvitamin D3 on human mesangial cells, Am. J. Kidney Dis. 18 (1991) 359–366.
[8] T. Weinreich, A. Muller, R.P. Wuthrich, C. Booy, U. Binswanger, 1,25-dihydroxyvitamin D3 and the synthetic vitamin D analogue, KH, Modulate the growth of mouse proximal tubular cells 1060, Kidney Blood Press. Res. 19 (1996) 325–331.
[9] N. Xing, M.L. Maldonado, L.A. Bachman, D.J. McKean, R. Kumar, M.D. Griffin, Distinctive dendritic cell modulation by vitamin D(3) and glucocorticoid pathways, Biochem. Biophys. Res. Commun. 297 (2002) 645–652.
[10] J. Tian, Y. Liu, L.A. Williams, D. de Zeeuw, Potential role of active vitamin D in retarding the progression of chronic kidney disease, Nephrol. Dial. Transplant. 22 (2007) 321–328.
[11] J.W. Park, E.H. Bae, I.J. Kim, S.K. Ma, C. Choi, J. Lee, S.W. Kim, Paricalcitol attenuates cyclosporine-induced kidney injury in rats, Kidney Int. 77 (2010) 1076–1085.
[12] J.W. Park, E.H. Bae, I.J. Kim, S.K. Ma, C. Choi, J. Lee, S.W. Kim, Renoprotective effects of paricalcitol on gentamicin-induced kidney injury in rats, Am. J. Physiol. Renal Physiol. 298 (2010) F301–F313.
[13] K. Makibayashi, M. Tatematsu, M. Hirata, N. Fukushima, K. Kusano, S. Ohashi, H. Abe, K. Kuze, A. Fukatsu, T. Kita, T. Doi, A vitamin D analog ameliorates glomerular injury on rat glomerulonephritis, Am. J. Pathol. 158 (2001) 1733–1741.
[14] M.D. Sanchez-Nino, M. Bozic, E. Cordoba-Lanus, P. Valcheva, O. Gracia, M. Ibarz, E. Fernandez, J.F. Navarro-Gonzalez, A. Ortiz, J.M. Valdivielso, Beyond proteinuria: VDR activation reduces renal inflammation in experimental diabetic nephropathy, Am. J. Physiol. Renal Physiol. 302 (2012) F647–F657.
[15] G. Xiang, T. Seki, M.D. Schuster, P. Witkowski, A.J. Boyle, F. See, T.P. Martens, A. Kocher, H. Sondermeijer, H. Krum, S. Itescu, Catalytic degradation of vitamin D up-regulated protein 1 mRNA enhances cardiomyocyte survival and prevents left ventricular remodeling after myocardial ischemia, J. Biol. Chem. 280 (2005) 39394–39402.
[16] F. Ekici, B. Ozyurt, H. Erdogan, The combination of vitamin D3 and dehydroascorbic acid administration attenuates brain damage in focal ischemia, Neurol. Sci. 30 (2009) 207–212.
[17] M.M. Yassin, D.W. Harkin, A.A. Barros D'Sa, M.I. Halliday, B.J. Rowlands, Lower limb ischemia–reperfusion injury triggers a systemic inflammatory response and multiple organ dysfunction, World J. Surg. 26 (2002) 115–121.
[18] T. Miyaji, X. Hu, P.S. Yuen, Y. Muramatsu, S. Iyer, S.M. Hewitt, R.A. Star, Ethyl pyruvate decreases sepsis-induced acute renal failure and multiple organ damage in aged mice, Kidney Int. 64 (2003) 1620–1631.
[19] L. Verstrepen, T. Bekaert, T.L. Chau, J. Tavernier, A. Chariot, R. Beyaert, TLR-4, IL-1R and TNF-R signaling to NF-kappaB: variations on a common theme, Cell. Mol. Life Sci. 65 (2008) 2964–2978.
[20] H. Wu, J. Ma, P. Wang, T.M. Corpuz, U. Panchapakesan, K.R. Wyburn, S.J. Chadban, HMGB1 contributes to kidney ischemia reperfusion injury, J. Am. Soc. Nephrol. 21 (2010) 1878–1890.
[21] H. Wu, G. Chen, K.R. Wyburn, J. Yin, P. Bertolino, J.M. Eris, S.I. Alexander, A.F. Sharland, S.J. Chadban, TLR4 activation mediates kidney ischemia/reperfusion injury, J. Clin. Invest. 117 (2007) 2847–2859.
[22] M.T. Lotze, K.J. Tracey, High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal, Nat. Rev. Immunol. 5 (2005) 331–342.
[23] R. Medzhitov, P. Preston-Hurlburt, C.A. Janeway Jr., A human homologue of the Drosophila Toll protein signals activation of adaptive immunity, Nature 388 (1997) 394–397.
[24] F. Chen, V. Castranova, X. Shi, New insights into the role of nuclear factor-kappaB in cell growth regulation, Am. J. Pathol. 159 (2001) 387–397.
[25] J.I. Lee, G.J. Burckart, Nuclear factor kappa B: important transcription factor and therapeutic target, J. Clin. Pharmacol. 38 (1998) 981–993.
[26] K. Sadeghi, B. Wessner, U. Laggner, M. Ploder, D. Tamandl, J. Friedl, U. Zugel, A. Steinmeyer, A. Pollak, E. Roth, G. Boltz-Nitulescu, A. Spittler, Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns, Eur. J. Immunol. 36 (2006) 361–370.
[27] C. Thota, T. Farmer, R.E. Garfield, R. Menon, A. Al-Hendy, Vitamin D elicits anti-inflammatory response, inhibits contractile-associated proteins, and modulates Toll-like receptors in human myometrial cells, Reprod. Sci. 20 (2013) 463–475.
[28] G. Murillo, V. Nagpal, N. Tiwari, R.V. Benya, R.G. Mehta, Actions of vitamin D are mediated by the TLR4 pathway in inflammation-induced colon cancer, J. Steroid Biochem. . Biol. 121 (2010) 403–407.
[29] F. Li, P. Liu, X. Zhang, Q. Zhang, S. Tang, M. Zhu, M. Qiu, 1,25(OH)D-mediated amelioration of aortic injury in streptozotocin-induced diabetic rats, Inflammation 36 (2013) 1334–1343.
[30] C.L. Roth, C.T. Elfers, D.P. Figlewicz, S.J. Melhorn, G.J. Morton, A. Hoofnagle, M.M. Yeh, J.E. Nelson, K.V. Kowdley, Vitamin D deficiency in obese rats exacerbates nonalcoholic fatty liver disease and increases hepatic resistin and Toll-like receptor activation, Hepatology 55 (2012) 1103–1111.
[31] H.S. Hwang, K.J. Yang, K.C. Park, H.S. Choi, S.H. Kim, S.Y. Hong, B.H. Jeon, Y.K. Chang, C.W. Park, S.Y. Kim, S.J. Lee, C.W. Yang, Pretreatment with paricalcitol attenuates inflammation in ischemia–reperfusion injury via the up-regulation of cyclooxygenase-2 and prostaglandin E2, Nephrol. Dial. Transplant. 28 (2013) 1156–1166.
[32] P.V. Ranganathan, C. Jayakumar, R. Mohamed, Z. Dong, G. Ramesh, Netrin-1 regulates the inflammatory response of neutrophils and macrophages, and suppresses ischemic acute kidney injury by inhibiting COX-2-mediated PGE2 production, Kidney Int. 83 (2013) 1087–1098.
[33] L.J. Crofford, B. Tan, C.J. McCarthy, T. Hla, Involvement of nuclear factor kappa B in the regulation of cyclooxygenase-2 expression by interleukin-1 in rheumatoid synoviocytes, Arthritis Rheum. 40 (1997) 226–236.
[34] M.P. Charalambous, T. Lightfoot, V. Speirs, K. Horgan, N.J. Gooderham, Expression of COX-2, NF-kappaB-p65, NF-kappaB-p50 and IKKalpha in malignant and adjacent normal human colorectal tissue, Br. J. Cancer 101 (2009) 106–115.
[35] K. Furuichi, T. Wada, H. Yokoyama, K.I. Kobayashi, Role of cytokines and chemokines in renal ischemia–reperfusion injury, Drug News Perspect. 15 (2002) 477–482.
[36] J.C. Rice, J.S. Spence, D.L. Yetman, R.L. Safirstein, Monocyte chemoattractant protein-1 expression correlates with monocyte infiltration in the post-ischemic kidney, Ren. Fail. 24 (2002) 703–723.
[37] M.L. Kielar, R. John, M. Bennett, J.A. Richardson, J.M. Shelton, L. Chen, D.R. Jeyarajah, X.J. Zhou, H. Zhou, B. Chiquett, G.T. Nagami, C.Y. Lu, Maladaptive role of IL-6 in ischemic acute renal failure, J. Am. Soc. Nephrol. 16 (2005) 3315–3325.
[38] D.K. de Vries, J.H. Lindeman, D. Tsikas, E. de Heer, A. Roos, J.W. de Fijter, A.G. Baranski, J. van Pelt, A.F. Schaapherder, Early renal ischemia–reperfusion injury in humans is dominated by IL-6 release from the allograft, Am. J. Transplant. 9 (2009) 1574–1584.
[39] X. Tan, X. Wen, Y. Liu, Paricalcitol inhibits renal inflammation by promoting vitamin D receptor-mediated sequestration of NF-kappaB signaling, J. Am. Soc. Nephrol. 19 (2008) 1741–1752.