



ORIGINAL ARTICLE

Disrupted tubular parathyroid hormone/parathyroid hormone receptor signaling and damaged tubular cell viability possibly trigger postsurgical kidney injury in patients with advanced hyperparathyroidism

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ABSTRACT

Background. Parathyroidectomy (PTX) that alleviates clinical manifestations of advanced hyperparathyroidism, including hypercalcemia and hypophosphatemia, is considered the best protection from calcium overload in the kidney. However, little is known about the relationship between postsurgical robust parathyroid hormone (PTH) reduction and perisurgical renal tubular cell viability. Post-PTX kidney function is still a crucial issue for primary hyperparathyroidism (PHPT) and tertiary hyperparathyroidism after kidney transplantation (THPT).

Methods. As a clinical study, we examined data from 52 consecutive patients (45 with PHPT, 7 with THPT) who underwent PTX in our center between 2015 and 2017 to identify post-PTX kidney injury. Their clinical data, including urinary liver-type fatty acid-binding protein (L-FABP), a tubular biomarker for acute kidney injury (AKI), were obtained from patient charts. An absolute change in serum creatinine level of 0.3 mg/dL (26.5 μ mol/L) on Day 2 after PTX defines AKI. Post-PTX calcium supplement dose adjustment was performed to strictly maintain serum calcium at the lower half of the normal range. To mimic post-PTX-related kidney status, a unique parathyroidectomized rat model was produced as follows: 13-week-old rats underwent thyroparathyroidectomy (TPTX) and/or 5/6 subtotal nephrectomy (NX). Indicated TPTX rats were given continuous infusion of a physiological level of 1–34 PTH using a subcutaneously implanted osmotic minipump.

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Immunofluorescence analyses were performed by polyclonal antibodies against PTH receptor (PTHr) and a possible key modulator of kidney injury, Klotho.

Results. Patients' estimated glomerular filtration rate (eGFR) did not have any clinically relevant change (62.5 ± 22.0 versus 59.4 ± 21.9 mL/min/1.73 m², NS), whereas serum calcium (2.7 ± 0.18 versus 2.2 ± 0.16 mmol/L, $P < 0.0001$) and phosphorus levels (0.87 ± 0.19 versus 1.1 ± 0.23 mmol/L, $P < 0.0001$) were normalized and PTH decreased robustly (181 ± 99.1 versus 23.7 ± 16.8 pg/mL, $P < 0.0001$) after successful PTX. However, six patients who met postsurgical AKI criteria had lower eGFR and greater L-FABP than those without AKI. Receiver operating characteristics (ROC) analysis revealed eGFR < 35 mL/min/1.73 m² had 83% accuracy. Strikingly, L-FABP > 9.8 μg/g creatinine had 100% accuracy in predicting post-PTX-related AKI. Rat kidney PTHr expression was lower in TPTX. PTH infusion (+PTH) restored tubular PTHr expression in rats that underwent TPTX. Rats with TPTX, +PTH and 5/6 NX had decreased PTHr expression compared with those without 5/6 NX. 5/6 NX partially cancelled tubular PTHr upregulation driven by +PTH. Tubular Klotho was modestly expressed in normal rat kidneys, whereas enhanced patchy tubular expression was identified in 5/6 NX rat kidneys. This Klotho and expression and localization pattern was absolutely canceled in TPTX, suggesting that PTH indirectly modulated the Klotho expression pattern. TPTX +PTH recovered tubular Klotho expression and even triggered diffusely abundant Klotho expression. 5/6 NX decreased viable tubular cells and eventually downregulated tubular Klotho expression and localization.

Conclusions. Preexisting tubular damage is a potential risk factor for AKI after PTX although, overall patients with hyperparathyroidism are expected to keep favorable kidney function after PTX. Patients with elevated tubular cell biomarker levels may suffer post-PTX kidney impairment even though calcium supplement is meticulously adjusted after PTX. Our unique experimental rat model suggests that blunted tubular PTH/PTHr signaling may damage tubular cell viability and deteriorate kidney function through a Klotho-linked pathway.

Keywords: hyperparathyroidism, Klotho, parathyroidectomy, PTH receptor, tubular injury

INTRODUCTION

Parathyroidectomy (PTX) is a recommended option for primary hyperparathyroidism (PHPT) [1, 2], especially in patients with chronic kidney disease, because kidney injury, a common comorbidity of PHPT, has long been regarded as an indication for surgery [3–6]. Tertiary hyperparathyroidism (THPT), followed by successful kidney transplantation, has clinical manifestations in common with PHPT, including hypercalcemia and hypophosphatemia [7, 8], the latter of which is a completely different feature from secondary hyperparathyroidism (SHPT), where phosphorus retention is frequently observed [9].

The association between hyperparathyroidism and kidney tubular injury has been the focus of discussion for the past 2 decades [10–14], primarily because parathyroid hormone (PTH) directly enhances calcium reabsorption in the cortical thick ascending limb of the loop of Henle, the distal convoluted tubule and the cortical collecting tubule in the kidney [15]. In addition, autonomously elevated PTH levels indirectly contribute to the calciuresis, acting via the calcium-sensing receptor [16, 17].

Clinically, PTX that alleviates various hyperparathyroidism-related complications such as hypercalcemia is considered the best protection from calcium overload in the kidney [18]. It is, however, still a controversial issue whether PTX exclusively has postsurgical benefits in terms of preserving kidney function [19]. We hypothesized that a steep decline of PTH in and of itself affects tubular cell stability and its function after PTX.

The present study was designed to address this concerning issue by introducing both clinical and experimental approaches: a single-center retrospective cohort study of consecutive patients with PHPT or THPT undergoing PTX, focusing on preexisting kidney tubular injury and postsurgical kidney function; and an investigation of pathophysiological findings of kidney samples using an experimental parathyroidectomized rat model. With this experimental model, we examined the possible involvement of the PTH receptor (PTHr) [20] and Klotho expression in tubular cells following parathyroid surgical ablation.

MATERIALS AND METHODS

Clinical cohort study

Patients and biochemical assays. We examined 52 consecutive patients with advanced PHPT or THPT who underwent PTX in our center between June 2015 and May 2017. The study was approved by the local ethics committee (Nagoya Daini Red Cross Hospital) and performed in accordance with the Declaration of Helsinki.

Blood and urine samples were collected before PTX and on Day 2 after PTX. Routine assays were performed and calculated, including corrected calcium (calcium), phosphorus, creatinine and albumin unless otherwise mentioned. Intact PTH was analyzed using electrochemiluminescence immunoassay (IMMULITE 2000, Siemens Healthneers, Tokyo, Japan). Urinary liver-type fatty acid-binding protein (L-FABP), which is expressed in kidney tubules at higher levels when the tubular cells are affected by ischemia or oxidative stress, was measured by a latex agglutination turbidimetry procedure (Sekisui Medical, Tokyo, Japan) as a potential kidney injury biomarker. L-FABP is known to be present in higher levels in excreted urine when tubular injury occurs [21].

Calcium supplementation protocol after PTX

Postsurgical calcium supplementation was meticulously designed and performed by administering alfacalcidol and calcium carbonate, with the aim to maintain serum calcium in the lower half of the normal range [22].

Postsurgical kidney function

We calculated estimated glomerular filtration rate (eGFR) by the Modification of Diet in Renal Disease equation before PTX and on Day 2 after PTX. Acute kidney injury (AKI) criteria after PTX were adopted according to the Kidney Disease: Improving Global Outcomes guideline stating that an absolute change in serum creatinine level of 0.3 mg/dL (26.5 μmol/L) defines AKI [23].

Graft kidney histology

Protocol graft kidney biopsies were obtained from transplant recipients with THPT according to standard renal biopsy techniques. Briefly, 3- μ m thick serial sections were stained with hematoxylin and eosin, periodic acid-Schiff, Masson's trichrome and periodic acid methenamine silver [24].

Statistical analyses

Data are expressed as mean \pm standard deviation (SD) or median [interquartile range (IQR)] with nonparametric distributed values. Differences before and after PTX were determined using paired t-test and group comparisons were carried out using Mann-Whitney *U* test. A receiver operating characteristics (ROC) curve was plotted to compare the diagnostic performance of eGFR and L-FABP. All statistical analyses were performed using SPSS for Windows version 13.0 (IBM, Chicago, IL, USA) and EZR version 1.36 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [25].

In vivo experiment to mimic sharp reduction of PTH and analyze kidney pathophysiology

Experimental model. A rat model of hypoparathyroidism with or without chronic kidney disease was produced using the methods described elsewhere [26]. Briefly, 13-week-old male Sprague Dawley rats weighing 350 g underwent thyroparathyroidectomy (TPTX) and/or 5/6 nephrectomy (NX). A group that underwent TPTX alone was also included. Indicated TPTX rats were administered a continuous infusion of a physiological level of 1-34 PTH (0.1 μ g/kg/h; Peninsula Laboratories, Talyo Way, San Carlos, CA, USA) using a subcutaneously implanted Alzet osmotic minipump (Model 2002; Alza, Palo Alto, CA, USA; pumps exchanged every 2 weeks) and subcutaneous L-thyroxin (Sigma Chemical, St Louis, MO, USA) at 4 μ g/kg body weight thrice weekly, beginning on the second day after TPTX. All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Immunofluorescence

Polyclonal antibodies against PTHR and Klotho were purchased from Abcam (Cambridge, UK). Immunohistochemical fluorescence is described elsewhere [27]. Briefly, immunoglobulin G purified from antiserum was labeled with a biotinylation kit (GE Healthcare, Little Chalfont, UK). Primary antibody-conjugated secondary antibodies were purchased from Life Technologies (Carlsbad, CA, USA). Metalloproteinase inhibitor (BB-94) was purchased from Tocris Bioscience (Ellisville, MO, USA).

RESULTS

Clinical cohort data before and after PTX

Table 1 presents baseline characteristics of our entire cohort. Most patients had sporadic PHPT, while two patients had multiple endocrine neoplasia type 1 and seven patients had THPT.

Postsurgically, serum-corrected calcium levels decreased and phosphorus levels increased (Table 2), while PTH had a robust decrease, indicating PTX was successfully performed. No clinically relevant change in eGFR was observed before and after PTX. No significant change was identified in L-FABP, β 2 microglobulin or albumin excretion ratio.

Table 1. Baseline characteristics in 52 patients who underwent PTX

Characteristics	Value
Sex (male/female), <i>n</i>	13/39
Age (years), mean \pm SD	59 \pm 14
Hyperparathyroidism, <i>n</i>	
Sporadic	43
MEN1	2
Tertiary	7

MEN1, multiple endocrine neoplasia type 1.

Table 2. Patient blood and urine biochemistry before and after PTX

Variables	Before PTX	Day 2 after PTX	P-value
Serum calcium (mmol/L)	2.7 \pm 0.18	2.2 \pm 0.16	<0.0001
Serum phosphorus (mmol/L)	0.87 \pm 0.19	1.1 \pm 0.23	<0.0001
eGFR (mL/min/1.73m ²)	62.5 \pm 22.0	59.4 \pm 21.9	NS
Serum intact PTH (pg/mL)	181 \pm 99.1	23.7 \pm 16.8	<0.0001
Urinary L-FABP (mg/gCr)	2.37 (1.23–4.55)	2.82 (1.85–4.04)	NS
Urinary β 2m (mg/gCr)	102 (62.7–319)	139 (90.6–269)	NS
UACR (mg/gCr)	11.3 (6.60–30.0)	8.95 (4.70–20.0)	NS

Data are given as mean \pm SD or median (IQR). P-values represent differences between before and after PTX.

Calcium, corrected calcium; gCr, grams creatinine; β 2m, β 2 microglobulin; UACR, urine albumin:creatinine ratio; NS, not significant.

AKI prevalence and accurate diagnostic values

In our clinical cohort, six patients eventually met AKI criteria after PTX (Figure 1A and B). They had comparable corrected calcium and phosphorus levels, but higher intact PTH and lower eGFR than those without AKI before PTX (Figure 1C and D). Strikingly, L-FABP levels were robustly elevated in patients with AKI (Figure 1E), suggesting that subclinical renal tubular injury may cause acute kidney impairment after PTX. The ROC curve (Figure 2) indicated that eGFR before PTX <35 mL/min/1.73 m² had an area under the curve (AUC) of 0.83 (83% diagnostic accuracy) to predict AKI after PTX. Surprisingly, the AUC of L-FABP >9.8 μ g/g creatinine had perfectly superior accuracy [1.0 (100%)] to diagnose postsurgical AKI.

Graft biopsy-confirmed preexisting tubular damage

Our cohort included a kidney transplant recipient who underwent graft kidney biopsy before PTX. Histology revealed moderate streaky interstitial fibrosis and tubular atrophy was moderately observed, resulting in AKI after PTX (Figure 3). In contrast, all intraglomerular structures were fairly well preserved without mesangial expansion.

Rat kidney immunoreactivity for PTHR and Klotho

Immunofluorescence staining for PTHR and Klotho had an interesting expression pattern in each treatment group. Largely consistent with expression of PTHR, modest expression was observed in proximal tubules in the control group (Figure 4A), whereas lower expression was seen in the group with 5/6 subtotal NX, where patchy tubular enlargement and degeneration were seen as expected (Figure 4B). Rat kidney with TPTX alone

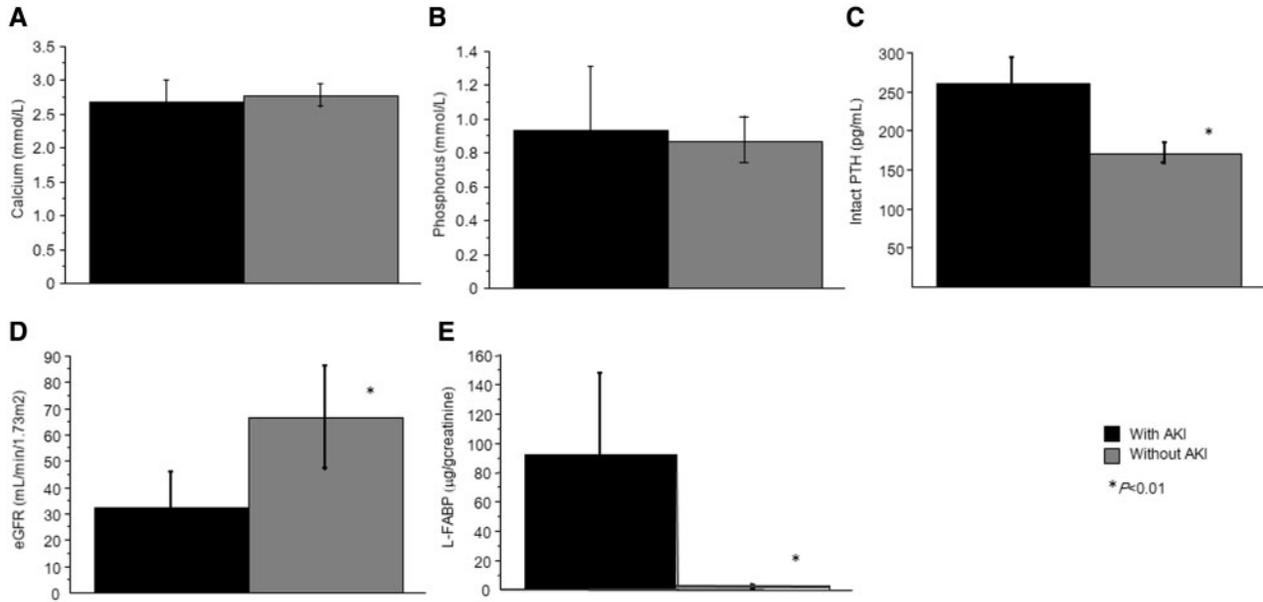


FIGURE 1: Presurgical mean representative variables with or without AKI. Calcium, corrected calcium. Black bar, before PTX; gray bar, after PTX. Data are presented as mean \pm SD. * $P < 0.01$ between with and without AKI.

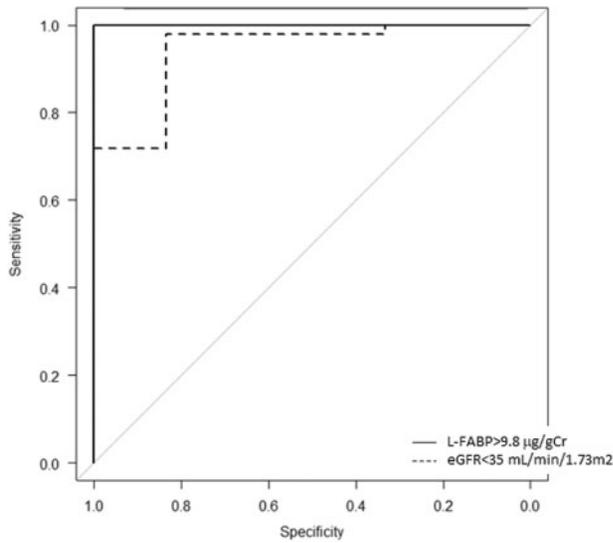


FIGURE 2: ROC analyses of two different diagnostic parameters from predicting AKI after PTX. ROC curve calculated to compare the accuracy of eGFR (dashed line) and L-FABP (solid line). The cutoff point of each variable to best match the prevalence of AKI was 35 mL/min/1.73 m² (eGFR), 9.8 µg/g creatinine (L-FABP).

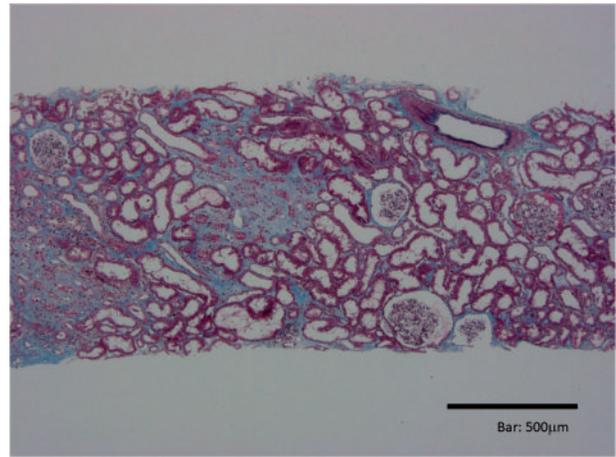


FIGURE 3: Preexisting tubular damage verified by graft kidney biopsy in a transplant recipient with advanced hyperparathyroidism. Histopathological features of a representative graft kidney specimen show moderate streaky interstitial fibrosis and tubular atrophy without marked glomerular abnormalities.

NX (Figure 5E). Supplementary figures show characteristic immunofluorescence pattern of PTHR (Figure S1) and Klotho (Figure S2).

completely diminished PTHR expression in tubules (Figure 4C) compared with controls and/or 5/6 NX. PTH infusion after TPTX upregulated PTHR expression diffusely and abundantly in tubular cells (Figure 4D). These PTHR enhancements were decreased in enlarged degenerated tubules of rats with TPTX, PTH infusion and 5/6 NX (Figure 4E).

Klotho was localized in certain tubular cells in controls (Figure 5A). Its expression pattern was enhanced in 5/6 NX (Figure 5B). Surprisingly, the Klotho expression and localization pattern were completely altered in rats with TPTX (Figure 5C). PTH infusion reproduced a Klotho expression pattern similar to controls (Figure 5D). Viable upregulated Klotho-positive tubular cells were observed in rats with TPTX, PTH continuous infusion and 5/6

DISCUSSION

Our clinical study assessed the possibility of kidney function impairment after PTX, although our postsurgical calcium supplement regimen maintained well-balanced calcium levels after PTX without serious iatrogenic hypercalcemic events. Kidney functions of our overall cohort, including those of posttransplant graft kidney, did not change before and after PTX. One of the critical findings of our present study is that patients with potential tubular injury, indicated by elevated L-FABP, had a robustly higher incidence of postsurgical AKI. Furthermore, ROC analysis suggests that tubular injury marker can distinguish patients who could bear postsurgical AKI risk more specifically

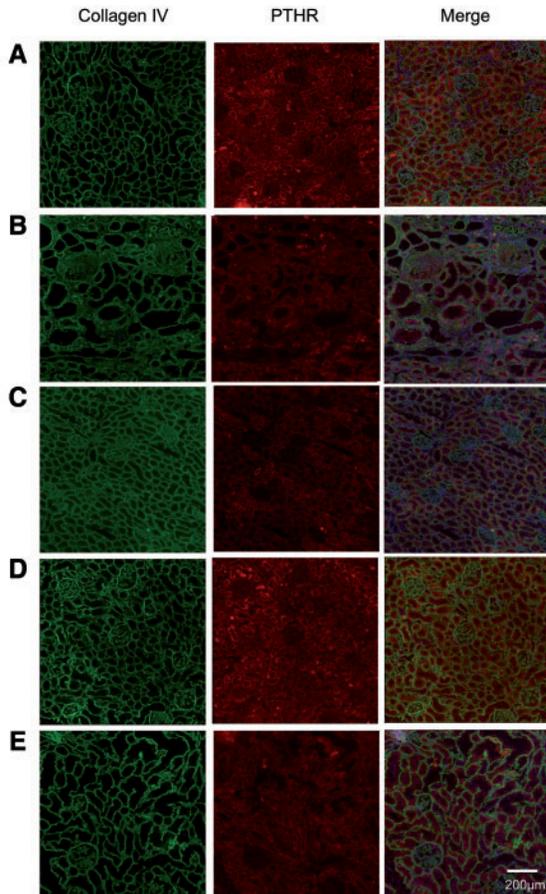


FIGURE 4: Immunoreactivity pattern of PTHR in experimental rat kidneys. Representative confocal images of kidney tissue samples: (A) control, (B) 5/6 NX, (C) TPTX, (D) TPTX + PTH infusion and (E) TPTX + PTH + 5/6 NX, immunohistostaining for collagen IV (green) and PTHR (red). Bar = 200 μ m.

than eGFR, whereas eGFR is a fair surrogate marker to predict kidney impairment. Presurgical elevated PTH levels together with marked hypercalcemia may potentiate tubular burden, especially in patients with lower tubular cell viability before PTX. The strength of our clinical findings is focusing on a tubular injury marker, L-FABP, because abnormally elevated PTH and calcium levels not only affect potential tubular cell function, but eventually potentiate an intratubular PTH-calcium milieu by robust PTH decline, which could also deteriorate kidney function after PTX. Other AKI markers, such as neutrophil-associated gelatinase lipocalin and kidney injury molecule-1, may also be associated with acute eGFR decline after PTX because these markers are sensitive to tubular cell damage rather than hemodynamic changes from a blood-lowering effect [28]. Our light microscopic findings from the kidney transplant recipient with THPT suggest that profound posttransplant tubular damage triggers AKI after PTX. Tubular cell viability may be linked to elevated tubular histological markers, such as L-FABP, resulting in kidney dysfunction after PTX. One of the crucial issues is that PTH alone influences tubular function. In tubular cells, PTH and PTHR synergistically work as a regulator of phosphorus and 1,25-dihydroxyvitamin D₃ levels by targeting sodium-phosphate cotransporter and 1 α hydroxylase. Our primitive hypothesis is whether PTH/PTHr signaling is associated with tubular cell stability and its function.

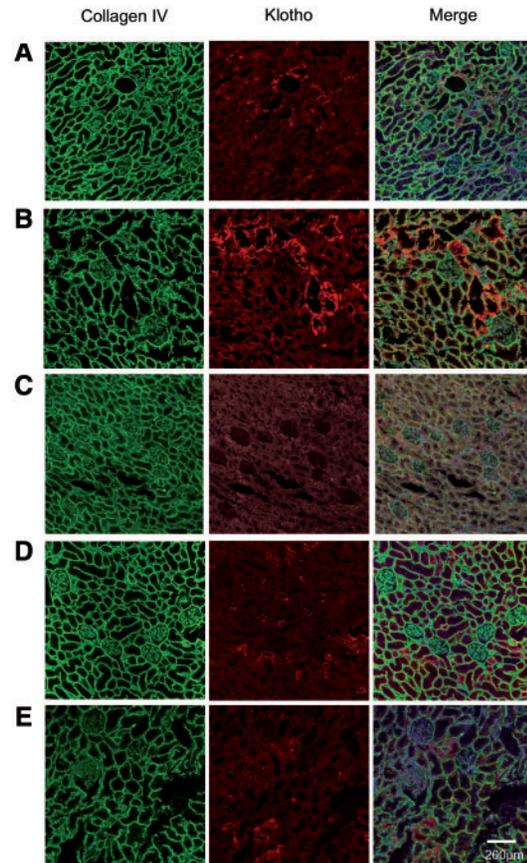


FIGURE 5: Immunoreactivity pattern of Klotho in experimental rat kidneys. Representative confocal images of kidney tissue samples: (A) control, (B) 5/6 NX, (C) TPTX, (D) TPTX + PTH infusion (PTH) and (E) TPTX + PTH + 5/6 NX immunohistostaining for collagen IV (green) and Klotho (red). Bar = 200 μ m.

It is possible that PTX may abolish the vasodilatory effect maintained by PTH/PTH-related protein, as both molecules can bind PTHR and modulate its renal vascular tone as a ligand [29–31]. A steep decline of PTH action on the renovascular system could lead to perivascular ischemia and cause irreversible interstitial tubule cell damage, as PTH-dependent PTHR upregulation [32] is unlikely to occur. Reduced PTHR expression may deteriorate renovascular resistance, in accordance with the findings in a hypertensive rat model [33]. The crucial limitation of our rat experiments is that the complexed mechanism of PTHR regulation was not fully examined, because PTH binding affinity to PTHR is diversely regulated depending on cell lines or glucocorticoid cotreatments [34].

The rat model that underwent TPTX was applied to clarify PTHR expression and distribution. Our *in vivo* experiment results indicate that ligand PTH ablation clearly downregulates PTHR expression, whereas PTH infusion restores its receptor expression and localization. Rats with 5/6 NX had lower PTHR expression in their tubular cells, suggesting that patients with PHPT or THPT may suffer critical stress caused by a steep decline of PTH, especially in the prevalence of chronic kidney disease. These findings do not completely suggest but partially support the idea that chronic kidney injuries decrease PTHR expression [35] and that surgical parathyroid resections maintain PTHR downregulation [36] in experimental rat models.

Klotho deficiency has been reported as a critical denominator for kidney disease progression [37–39]. Our experimental rat

kidney histologically demonstrates that rat kidney Klotho expression with 5/6 NX alone is markedly elevated in specific tubular cells, which supports the theory that Klotho is a counteracting tubular protective factor against kidney damage. Interestingly, surgical parathyroid ablation absolutely alters the Klotho expression pattern in tubular cells, and PTH administration restores the Klotho expression pattern to a similar extent in normal controls. These novel findings from *in vivo* experiments shed some light into the possible role of PTH/PTHrP signaling for maintaining kidney tubular cell viability through the Klotho-linked pathway. Its clinical relevance may become inevitable when patients with PHPT or THPT possess subclinical tubular cell damage before PTX.

There is no doubt that PTX restores hyperparathyroid-induced calcium imbalance and its clinical manifestations, including high turnover bone disease. In terms of renal involvement, several randomized controlled trials prove that PTX does not affect kidney function in patients with PHPT [40–43].

PTX should be performed properly since persistent hyperparathyroidism deteriorates kidney function [44]. In transplanted kidney, however, there is a long-standing controversy regarding whether PTX affects graft kidney function [45]. Calcimimetics may be an alternative treatment option. Indeed, we have examined several kidney transplant recipients with moderately well-controlled THPT using cinacalcet (Okada, *et al.*, unpublished data). Apparently their kidney function does not decline, whereas their serum calcium levels are within the upper half of the normal range (2.3–2.6 mmol/L), suggesting that they will need PTX when their calcium levels are elevated above the normal range. Interestingly, all of those transplant recipients with THPT had moderately progressive SHPT before kidney transplantation. Since there is still a lack of evidence about the advantage of PTX on kidney function in patients with preexisting tubular cell injury, calcimimetics may be a therapeutic tool to maintain PTH/calcium balance in patients concomitantly having subclinical kidney diseases with advanced hyperparathyroidism.

From both clinical and experimental studies, we strongly emphasize that successful PTX on postsurgical kidney function definitely depends on precise evaluation of tubular cell function. Patients with potential tubular cell damage may suffer kidney impairment, even though calcium supplement is meticulously adjusted after PTX, presumably due to a halted PTH/PTHrP cascade. In our unique rat experiments, postsurgical PTH administration recovered tubular PTHrP localization, which coordinately reestablished the Klotho expression pattern in normal controls. Further clinical study is needed to clarify whether postsurgical PTH administration helps prevent possible kidney damage after PTX.

In conclusion, elevated tubular injury marker levels more precisely predict postsurgical kidney impairment in patients with hyperparathyroidism than eGFR does. Disrupted PTH/PTHrP signaling may potentiate acute tubular cell damage by a certain mechanism where the tubular Klotho expression pattern is critically disturbed.

SUPPLEMENTARY DATA

Supplementary data are available at [ckjonline](http://ckjonline.com).

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AUTHORS' CONTRIBUTIONS

T.S. designed these two studies, analyzed data and wrote the article. Y.K. and Y.T. performed immunofluorescence of rat kidney specimens. S.Y. designed the experimental rat model and collected kidney tissue samples. J.J.K. organized the experimental rat model. Y.T., T.I., M.O. and T.H. performed PTX and managed postsurgical calcium supplementation. M.F. supervised both studies and examined the entire study concept. Conception, design, data collection and analysis as well as writing of this article were performed by investigators with no support from pharmaceutical companies.

CONFLICT OF INTEREST STATEMENT

M.F. has received honoraria from Kyowa Hakko Kirin and Ono Pharmaceutical for paid advisory boards. None of these activities had any influence on the results or interpretations in this article. The results presented in this article have not been published previously in whole or part, except in abstract form as the 54 th ERA-EDTA Congress, Madrid, Spain, 2017.

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