PTH induces bone endothelial–adipocyte transition in rats with chronic kidney disease of low bone mineral density

Li-Hua Ni; Ri-Ning Tang; Kai-Yun Song; Li-Ting Wang; Bi-Cheng Liu
Instutute of Nephrology, Zhongda Hospital, Sourthest University School of Medicine, Nanjing, China

Introduction and objectives

Low bone mineral density is one of the common complications in chronic kidney disease (CKD). It is an important risk factor of cardiovascular mortality among patients with CKD. However, its exact mechanism has not been completely elucidated. Recent studies indicated that the endothelial-to-mesenchymal transition (EndMT) of endothelial cells to adipocytes contributed to the development of low bone mineral density . Our preliminary studies have found that PTH could induce the phenotypic change of the endothelial cells in a dose and time-dependent manner and EndMT occurred at an early stage of CKD in animal models. In the present study, we aim to further clarify the mechanism of low bone mineral density in CKD by investigating the effects of PTH on EndMT of endothelial cells in the bone marrow . Our study will provide novel insights into the development of low bone mineral density in CKD and might contribute to the establishment of new strategy to its prevention.

Methods

Eight-week–old male Sprague Dawley rats were randomly divided into three groups: a control group (CTL; n=10), a vehicle-treated group (CKD; n=10) and a cinacalcet-treated group (CKD+CINA; n=10). CKD was induced by a 0.75% adenine diet for 4 weeks. After adenine withdrawal, all rats were maintained on a 1.5% phosphate diets for the next 30 weeks. At the initiation of the adenine diet, rats in the cinacalcet-treated group were orally administered cinacalcet (10mg/kg/d) for 34 weeks. We performed blood analysis, emission computed tomography (ECT), bone mechanical properties tests, dual energy X-ray absorptiometry (DEXA) and micro-computed tomography (micro-CT) assessment in rats at the age of 42 weeks. The expressions of EndMT, mesenchymal stem cell and adipocyte related markers in endothelial cells in the bone marrow were examined. The effects of elevated PTH on EndMT were also investigated in human bone marrow derived endothelial cells.

Results and conclusions

Compared with the CTL group, the levels of serum PTH, serum creatinine and blood urea nitrogen were significantly higher in the CKD group (Table1, P <0.05). Cinacalcet treatment decreased the levels of serum PTH, phosphate (P), and Ca (Calcium) × P product (Table 1, P <0.05). The ECT images of the parathyroid glands indicated hyperparathyroidism in CKD rats but normal parathyroid function in CTL rats and CKD+CINA rats (Figure1). Trabecular BV/TV, trabecular number, cortical area and cortical thickness measured by micro-CT in femur and lumbar vertebra were decreased in the CKD group, which were alleviated in the CKD+CINA group (Table 2, Figure 2, P <0.05). Force and stiffness of lumbar vertebra were decreased in CKD rats, which were attenuated in the CKD+CINA rats (Table 3, P <0.05). The bone mineral density (BMD) of femur and lumbar assessed by DEXA were decreased in the CKD group, which were attenuated in the CKD+CINA group (Table 4, P <0.05). The adipocytes infiltration in the CKD group by oil red O staining in femur were significantly increased compared with the CTL group, which were improved in cinacalcet treatment (Figure 3, P<0.05). The expression of endothelial marker CD31 was significantly downregulated in CKD rats, whereas expression of the mesenchymal marker fibroblast specific-protein 1 (FSP 1), mesenchymal stem markers (STRO-1, CD 44, CD 10), and adipocytes markers were markedly upregulated. These changes were inhibited by CINA treatment (Figure 4, 5, 6). These findings suggest a decline of BMD in uremic rats, which could be attenuated by cinacalcet treatment. PTH may lower BMD via inducing endothelial-adipocyte transition in CKD rats.

Table. 1. Physical and biochemical parameters of animal groups.

analyte	CTL (n=10)	CKD(n=10)	CKD+CINA(n=10)
body weigh (g)	493.30±45.26	383.30±59.71*	395.10±23.69*
serum creatine (Scr, mg/dl)	61.50±16.65	473.30±106.80*	424.90±46.15*
blood urea nitrogen(BUN, mg/dl)	188.90±44.16	547.50±119.60*	512.40±97.56*
Phosphorus (mmol/l)	2.41±0.08	3.81±0.27*	2.94±0.47*#
Calcium (mmol/l)	2.24±0.04	1.95±0.08*	1.99±0.11*
calcium×phosphorus ($mmol^2/l^2$)	5.38±0.20	7.42±0.51*	6.23±1.19*#
PTH (pg/ml)	71.50±13.77	517.60±86.6*	350.10±85.94*#

Figure. 1. The ECT of parathyroid glands in rats.

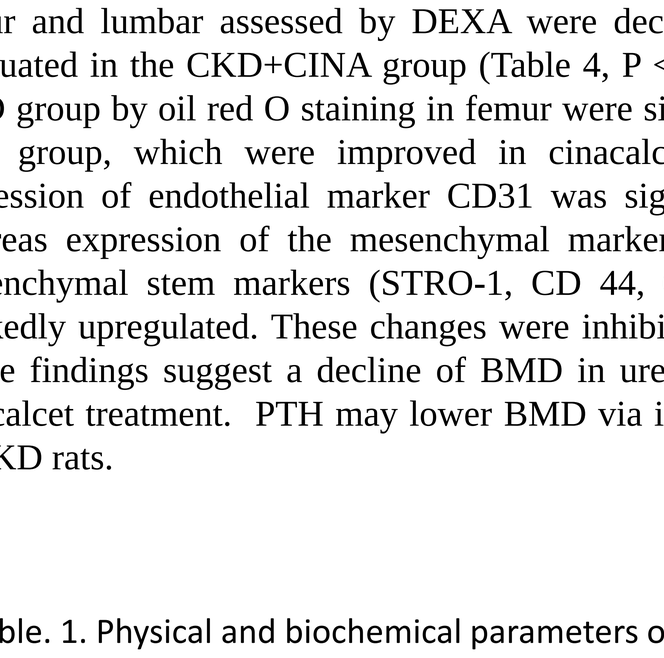


Table. 2. Micro CT bone parameters.

	CTL (n=10)	CKD(n=10)	CKD+CINA(n=10)
lumbar vertebra			
BV/TV(%)	15.562±3.498	8.494±1.397*	12.113±2.515*#
Tb.Th(mm)	0.145±0.036	0.094±0.016*	0.115±0.019*
Tb.N(1/mm)	1.509±0.249	0.901±0.167*	1.212±0.223*#
Tb.Sp(mm)	0.693±0.148	1.105±0.158*	0.899±0.153*#
Ct.Th(mm)	0.149±0.024	0.126±0.020*	0.144±0.010#
distal femur			
BV/TV(%)	10.766±1.566	5.619±0.987*	8.625±1.932*#
Tb.Th(mm)	0.117±0.039	0.070±0.017*	0.086±0.114*
Tb.N(1/mm)	2.185±0.551	1.283±0.175*	1.691±0.218*#
Tb.Sp(mm)	0.509±0.134	1.131±0.245*	0.906±0.124*#
femoral diaphysis			
Ct.Th(mm)	0.487±0.043	0.419±0.054*	0.463±0.026#
Ct.Ar(mm^2)	4.986±0.801	4.053±0.536*	4.609±0.790#

Figure. 2. Micro CT images of femur and lumbar vertebra.

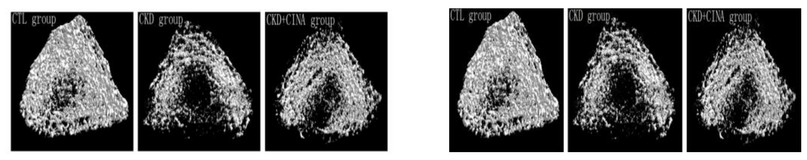


Table. 3. bone mechanics of lumbar vertebra.

	CTL(n=5)	CKD(n=5)	CKD+CINA(n=5)
Force(N)	307.66±44.04	249.85±38.04*	264.53±33.42*#
Displacement(mm)	0.73±0.13	0.66±0.15	0.70±0.11
Work to failure(mJ)	105.55±29.07	50.73±11.56*	47.60±9.01*
Stiffness(N/mm)	902.69±137.22	562.92±201.64*	739.67±91.97*#
Maximum stress(MPa)	40.14±9.28	37.08±19.70	39.82±12.88
Maximum strain(MPa)	0.14±0.02	0.12±0.04	0.13±0.01
Toughness(MPa)	2.22±0.68	2.17±0.69	2.09±0.53
Elastic modulus(MPa)	696.03±112.71	282.13±38.65*	351.12±84.33*

Table. 4. BMD by DEXA

BMD g/cm^2	CTL(n=10)	CKD(n=10)	CKD+CINA(n=10)
femur	0.256±0.022	0.216±0.021*	0.240±0.023#
vertebra lumbar	0.362±0.040	0.291±0.034*	0.324±0.016*#

Figure. 3. Oil red O staining of distal femur.

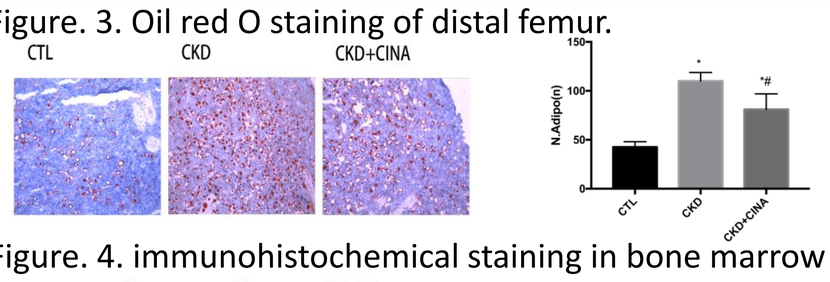


Figure. 4. immunohistochemical staining in bone marrow tissue

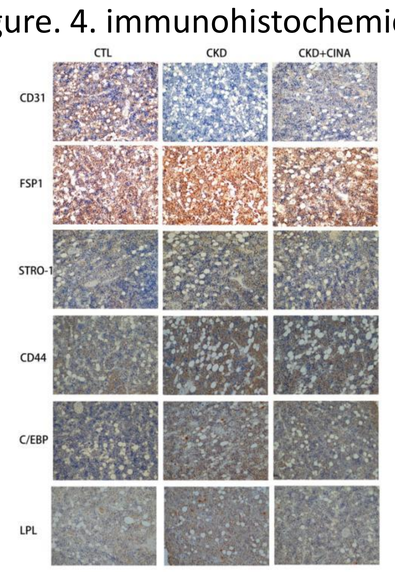


Figure. 5. qPCR analysis in bone marrow tissue.

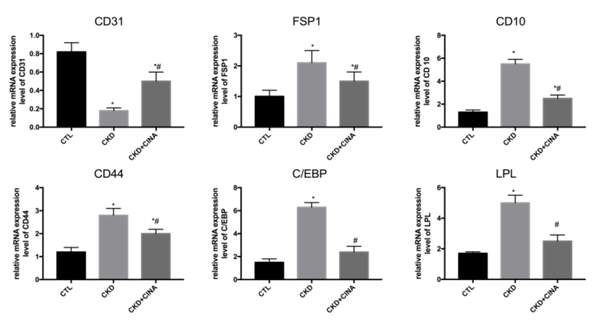
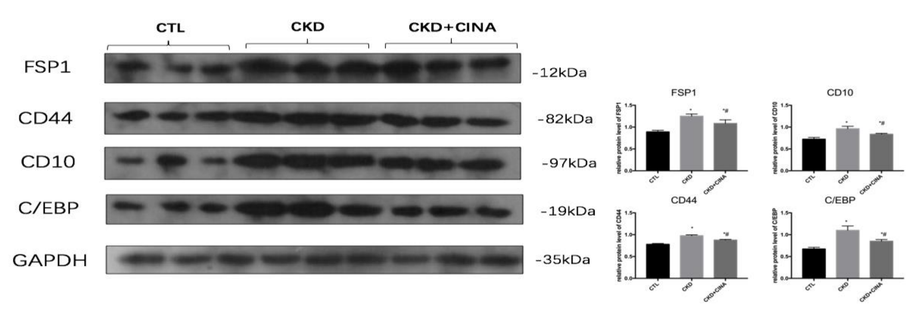


Figure. 6. Western blot analysis in bone marrow tissue.



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Li-Hua Ni
DOI: 10.3252/pso.eu.54ERA.2017
ePosters supported by F. Hoffmann- La Roche Ltd.
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