

Original Research Article

Effect of losartan potassium tablets combined with Bailing capsules on rats with chronic kidney disease

Wenhui Yu¹, Shuzhong Duan², Qiaorong Dong^{2*}

¹Department of Pharmacy, ²Department of Nephrology, Affiliated Hospital of Chengde Medical University, Chengde, Hebei 067000, China

*For correspondence: **Email:** dongqiaorong4@163.com; **Tel:** +86-13832406896

Sent for review: 3 April 2022

Revised accepted: 22 July 2022

Abstract

Purpose: To study the combined therapeutic effect of losartan potassium tablets and Bailing capsules in rats with chronic kidney disease (CKD) via PI3K/Akt/NF- κ B pathway.

Methods: Sixty Sprague Dawley (SD) rats were randomized into blank (BG), model (MG) and study groups (OG), with 20 rats in each group. The CKD model was established in MG and OG through oral gavage of adenine. The OG was administered losartan potassium tablets combined with Bailing capsules, while BG and MG were administered saline. After 6 weeks of continuous administration, BUN, Scr, UAib, TNF- α , IL-1 β and IL-6 were assessed. Protein expression and changes in the mRNA of PI3K, Akt and NF- κ B were determined.

Results: The BUN, Scr and UAib, as well as IL-1 β , IL-6 and TNF- α levels were highest in MG, followed by OG, and lowest in BG ($p < 0.05$). PI3K and Akt proteins were lowest in MG, followed by OG, and highest in BG, whereas NF- κ B protein was highest in MG, followed by OG, and lowest in BG ($p < 0.05$). PI3K mRNA and Akt mRNA levels were lowest in MG, followed by OG, and highest in BG, while NF- κ B mRNA was highest in MG, followed by OG, and lowest in BG ($p < 0.05$).

Conclusion: The combination of losartan potassium tablets and Bailing capsules are effective in treating CKD in rats, and improves the renal function of rats. These effects may be related to the down-regulation of PI3K/Akt/NF- κ B signaling pathway.

Keywords: Losartan, Bailing capsules, PI3K/Akt/NF- κ B pathway, Chronic kidney disease

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Chronic kidney disease (CKD) is a common disease and is often accompanied by manifestations such as hypertensive glomerulosclerosis or diabetic glomerulosclerosis [1]. Epidemiological studies show that the incidence of CKD in China is about 10.8 %, and the trend has been on the increase in recent

years, and could worsen in future. There are certain challenges regarding its prevention and treatment [2]. PI3K/Akt/NF- κ B is a key signaling pathway that regulates the transmission and expression of various signaling factors [3]. It has been reported that PI3K/Akt/NF- κ B signaling pathway affects the process of renal interstitial fibrosis and has close association with the occurrence of CKD [4].

Bailing capsules are a proprietary Chinese medicine made from fermented *Cordyceps* powder, which has some adjuvant effects on CKD and improves patients' renal function. Losartan potassium tablets are usually prescribed as antihypertensive drugs, which exerts renoprotective effects by antagonizing angiotensin receptors [5]. Some studies have shown that losartan potassium tablets combined with Bailing capsules can effectively treat CKD, significantly reduce clinical symptoms and promote the recovery of patients with high clinical value, but its mechanism of action is still unknown [6]. The objective of this study was to explore the combined effects of losartan potassium tablets and Bailing capsules in CKD rats.

EXPERIMENTAL

Laboratory animals

Sixty male Sprague Dawley (SD) rats, with body weight of 210 ± 10 g, were used in the animal experiments (license no. SYXY (Beijing, China) 2020-0038). They were housed in a sterile and clean environment with a constant temperature [12-h light/dark cycle, 23 ± 2 °C, 56 ± 3 % humidity, with access to free diet and water unrestricted movement. The rats were acclimatized for 7 days to adapt to the environment and avoid external stimuli.

Reagents and instruments

Losartan potassium tablets (Zhejiang Huahai Pharmaceutical Co. Ltd, no. H20070264 (Linhai, China); Bailing capsules (Qinghai Zhufeng Cordyceps Pharmaceutical Co. Ltd; no. Z20080187 (Qinghai, China); Adenine was purchased from Wuxi Jingyao Biotechnology Co. (Wuxi, China); Sterile normal saline was purchased from Weifang Zhonghui Co. Ltd. (Weifang, China); 10 % chloral hydrate was bought from Shanghai Shangbao Biotechnology Co. Ltd.; phosphate buffer was purchased from Ruichu Biotechnology Co. Ltd.; RIPA Buffer was bought from Qingdao Jieskang Co. Ltd. (Qingdao, China). Micropipette (VITLAB -164; Shanghai Chugong Industrial Co., Ltd., Shanghai, China); Surgical Instrument Kit (Yijianmei Medical Instrument Co., Ltd. (Yantai, China); high-speed cryogenic centrifuge was purchased from Sigma (St. Louis, USA); Infinite M100 PRO multifunctional microplate reader was purchased from TECAN (Shanghai, China); The AU5800 automatic biochemical analyzer was supplied by Beckman Coulter Co. Ltd (Franklin Lakes, NJ, USA); the protein electrophoresis

instrument was purchased from Bole; the fluorescence imaging system was purchased from Odyssey CLx (Shanghai, China); and the -80 °C refrigerator was purchased from Haier Co. Ltd. (Haier, China).

Grouping, modeling and drug administration

Sixty SD rats (SPF class) were randomized into blank (BG), model (MG) and observation groups (OG), with 20 rats in each group. The BG was not treated, and the CKD model was established via gavage of adenine in MG and OG, i.e., 2.5 % adenine suspension (300 mg/kg) was used for gavage and administered continuously for 4 weeks, and the rat CKD model was well established. The rats in OG were treated with Losartan potassium tablets (15 mg/kg) combined with Bailing capsules (0.625 g/kg). Both drugs were dissolved in saline and given via gavage, the dose of gavage given was 5 ml/kg once daily. BG and MG were treated with the same amount of normal saline for 6 weeks.

Ethical approval

This study was implemented after approval from the ethical committee of Affiliated Hospital of Chengde Medical University (approval no. LL2016086), and the experiments were carried out in conformity with Guide for the Care and Use of Laboratory Animals [7].

Evaluation of indices/parameters

Serum BUN, Scr and UAib

Two milliliters of arterial blood was obtained from the abdomen of the rats, placed in a centrifuge tube for 1 h, centrifuged (4 °C, 15 min, 12000 g), and the supernatant was obtained. The BUN, Scr, UAib levels and other renal function indices of the three groups were measured using an automatic biochemical analyzer.

IL-1 β , IL-6 and TNF- α

The parameter levels in the rat renal tissues were measured. The rat renal tissues were then taken, cleaned and made into homogenate, centrifuged (4 °C, 20 min, 15000 g), and the precipitate was discarded. The IL-1 β , IL-6 and TNF- α levels were determined by ELISA in strict accordance with the kit instructions.

PI3K, Akt and NF- κ B proteins

The PI3K, Akt, and NF- κ B proteins levels were measured using Western blotting. 100 microliters of pre-cooled RIPA buffer was added

to 1 mg of renal tissue for cryogenic grinding (the mortar was pre-cooled in liquid nitrogen in advance). Phosphatase inhibitor (10 μ L) and protease inhibitor (10 μ L) were added into 1 mL of pre-chilled RIPA buffer and mixed well before use. The samples were placed on a shaker platform at 4 °C and shaken for 1 h at medium speed (100 - 150 rpm), centrifuged at 14000 rpm at 4 °C for 20 min; they were then subjected to polyacrylamide gel electrophoresis: 15 % acrylamide gel electrophoresis, and 30 - 50 μ g protein loading. Wet transfer was performed for 30 - 100 min, followed by blocking with 5 % skimmed milk for 1 h at room temperature. Anti-PI3K, Akt, NF- κ B antibodies were added for incubation at 4 °C overnight. Goat anti-rabbit fluorescent secondary antibody was added and kept for 1 h of incubation. After PBST washing, PI3K, Akt, NF- κ B protein contents were measured by Odyssey infrared imaging system.

Changes in PI3K, Akt, NF- κ B mRNA

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to determine changes in PI3K, Akt, and NF- κ B mRNA. TRIzol method was used to extract RNA from cells after transfection for 48 h. MicroRNA Reverse Transcription Kit was used for cDNA synthesis, and fluorescence quantitative kit (SYBR Premix Ex Taq) was used for RT-PCR. The conditions are set as: pre-denaturation (90 °C, 5 min), denaturation (95 °C, 10 s), annealing (60 °C, 30 s), extension (70 °C, 30 s), for a total of 40 cycles. All the above operations were conducted strictly in accordance with the kit manufacturer's instructions (Table 1).

Statistical analysis

Data were processed using SPSS statistical analysis software (version 26.0). The measurement data are presented as mean \pm SD and compared by t-test between two groups. The count data are presented as %, and compared by χ^2 test. $P < 0.05$ represents statistically significance.

Table 1: Primer sequence

Primer	Sequence	
PI3K mRNA	Upstream	5'-CATCACTTCCTCCTGCTCTAT-3'
	Downstream	5'-CAGTTGTTGGCAATCTTCTT-3'
Akt mRNA	Upstream	5'-AAACCTGGCGGCCACGCTAC-3'
	Downstream	5'-TTGGCCAGGGCCACCTCCAT-3'
NF- κ B mRNA	Upstream	5'-CAAGATCTGCCGAGTAAAC-3'
	Downstream	5'-TCGGAACACAATGGCCACTT-3'

RESULTS

Renal functions of rats

The serum levels of BUN, Scr and UAlb in the OG rats were significantly elevated in comparison with the BG ($p < 0.05$). The levels in OG rats were significantly reduced in comparison with MG ($p < 0.05$) (Table 2).

Inflammatory mediators

The IL-1 β , IL-6 and TNF- α levels in the MG and OG were noticeably higher than those in the BG ($p < 0.05$). These levels in the OG were noticeably lower than those in MG ($p < 0.05$) (Table 3).

Protein expressions of PI3K, Akt and NF- κ B

MG exhibited noticeably lower PI3K and Akt protein levels and higher NF- κ B protein level than BG ($p < 0.05$); OG showed a significant decrease in the PI3K and Akt protein levels and a significant elevation in NF- κ B protein level after drug administration compared with BG ($p < 0.05$) (Table 4 and Figure 1).

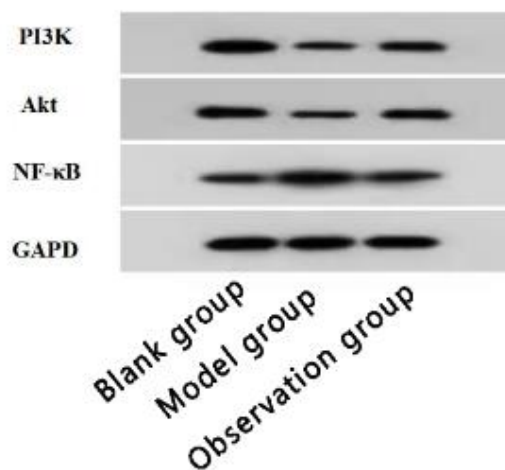


Figure 1: PI3K, Akt and NF- κ B protein expression

Table 2: Comparison of BUN, Scr, and UAib (mean \pm SD, n = 20)

Group	BUN (mmol/L)	Scr (μ mol/L)	UAib (mg/24h)
Blank	6.85 \pm 1.03	47.86 \pm 6.13	6.32 \pm 1.45
Model	15.37 \pm 2.94*	125.52 \pm 8.37*	11.72 \pm 2.53*
Study	7.26 \pm 1.12#	50.26 \pm 7.05#	6.69 \pm 1.57#

Note: compared with blank group, * p < 0.05; compared with model group, # p < 0.05

Table 3: Comparison of IL-1 β , IL-6 and TNF- α levels (mean \pm SD, n = 20, pg/mg)

Group	IL-1 β	IL-6	TNF- α
Blank	1.02 \pm 0.21	4.46 \pm 0.58	1.95 \pm 0.12
Model	3.15 \pm 0.32*	11.37 \pm 1.19*	3.38 \pm 0.37*
Study	1.03 \pm 0.17#	5.18 \pm 1.74#	2.04 \pm 0.25#

Note: compared with blank group, * p < 0.05; compared with model group, # p < 0.05

Table 4: Comparison of PI3K, Akt and NF- κ B protein levels (mean \pm SD, n = 20)

Group	PI3K	Akt	NF- κ B
Blank	0.85 \pm 0.27	0.76 \pm 0.25	0.30 \pm 0.02
Model	0.36 \pm 0.05*	0.41 \pm 0.12*	0.89 \pm 0.14*
Study	0.81 \pm 0.21#	0.72 \pm 0.23#	0.33 \pm 0.05#

Note: compared with blank group, * p < 0.05; compared with model group, # p < 0.05.

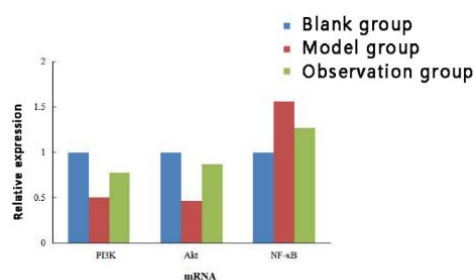
mRNA expressions of PI3K, Akt and NF- κ B

PI3K mRNA and Akt mRNA were significantly decreased while NF- κ B mRNA was significantly increased in the renal tissues in MG in contrast to BG (p < 0.05); PI3K mRNA and Akt mRNA were significantly decreased and NF- κ B mRNA was noticeably elevated in OG in contrast to BG (Table 5 and Figure 2).

Table 5: Comparison of the relative expression of PI3K, Akt and NF- κ B mRNA (mean \pm SD, n = 20)

Group	PI3K	Akt	NF- κ B
Blank	1.00 \pm 0.01	1.00 \pm 0.01	1.00 \pm 0.01
Model	0.51 \pm 0.06*	0.47 \pm 0.08*	1.56 \pm 0.26*
Study group	0.78 \pm 0.25#	0.87 \pm 0.27#	1.27 \pm 0.35#

Notes: compared with blank group, * P < 0.05; compared with model group, # P < 0.05

**Figure 2:** Comparison of the relative expression of PI3K, Akt and NF- κ B mRNA

DISCUSSION

The early stage of CKD is relatively insidious, but symptoms such as edema, hematuria and proteinuria may appear when the disease worsens [8]. It is also accompanied by irreversible renal fibrosis, which seriously affects the physiological function of the kidney and requires dialysis or symptomatic therapy in severe cases, affecting the life and health of patients [9]. Improvement in the prognosis and quality of life of CKD patients is one of the challenges in clinical practice [10]. The pathogenesis of CKD is complex, which is mainly related to abnormal signaling and energy metabolism disorders, accompanied by abnormal expression of various inflammatory factors [11]. Currently, there is still a lack of clinical cure for CKD, and it is important to elucidate the pathogenesis of CKD for its prevention and cure.

The PI3K/Akt/NF- κ B pathway participates in cell proliferation, differentiation, etc [12]. Upon reception of signals from external stimuli, PI3K on the cell membrane phosphorylates into PIP3, which activates Akt proteins. Downregulation of this pathway will damage renal tubular cells and affect kidney function [13]. The PI3K/Akt regulates downstream proteins involved in cellular regulation, among which NF- κ B is a key downstream factor of the PI3K/Akt pathway, and inhibition of this pathway activates the NF- κ B complex, leading to a massive release of inflammatory mediators and inducing an inflammatory response [14]. The NF- κ B protein expression was significantly increased, suggesting the close association of the PI3K/Akt/NF- κ B pathway with CKD.

The results of this study show that BUN, Scr and UAib were significantly elevated in MG when compared with BG. This indicates that the CKD rat model was successfully established and can be used for subsequent studies. In the present study, the CKD rat model was established by the administration of adenine via gavage, and the model is stable and reliable. The BUN, Scr and UAib levels of the rats in OG after drug intervention decreased significantly. This indicates that the combined therapy of losartan potassium tablets and Bailing capsules can greatly improve renal function indices and promote the recovery of renal function in rats with CKD [15,16]. This is mainly related to the effects of losartan potassium tablets which improves renal blood flow, while Bailing capsules have the effect of strengthening the spleen, thus benefiting the kidney and tonifying the qi; this helps to promote the recovery of patients.

The MG and OG showed noticeably increase in IL-1 β , IL-6 and TNF- α levels when compared with the BG. The inflammatory response in CKD rats increased, and so also the expressions of inflammatory mediators. OG exhibited significantly lower levels of IL-6, TNF- α , and IL-1 β than MG.

This is mainly related to the effect of Bailing capsules in regulating and enhancing the immunity of the organism [17]. It has been found that inhibition of PI3K/Akt pathway leads to excessive activation of NF- κ B, which induces massive release of inflammatory mediators [18].

CONCLUSION

Combining losartan potassium tablets with Bailing capsules is effective in treating rats with CKD. The mechanism of action may be linked to the downregulation of PI3K/Akt/NF- κ B signaling, which delays the progression of CKD. This finding provides a theoretical basis for the clinical application of losartan potassium tablets combined with Bailing capsules in CKD, and thus may be beneficial to the treatment and rehabilitation of patients with CKD.

DECLARATIONS

Acknowledgements

None provided.

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. Carrero JJ, Hecking M, Chesnaye NC, Jager KJ. Sex and gender disparities in the epidemiology and outcomes of chronic kidney disease. *Nat Rev Nephrol* 2018; 14(3): 151-164.
2. Feng Y, Liang W, Liang W, Xiao X, Zhang Y. Effects of XueZhiTong capsules on chronic kidney disease patients with dyslipidemia. *Trop J Pharm Res* 2022; 21(1):177-183 doi: 10.4314/tjpr.v21i1.26
3. He X, Jiang H, Gao F, Liang S, Wei M, Chen L. Indoxyl sulfate-induced calcification of vascular smooth muscle cells via the PI3K/Akt/NF- κ B signaling pathway. *Microsc Res Tech* 2019; 82(12): 2000-2006.
4. Lin H, Chen C. Suppressive effect of Hsa-miR-125a-5p on cervical cancer proliferation and invasion via regulation of Rab25-PI3K/AKT pathway. *Trop J Pharm Res* 2022; 21(2):221-227 doi: 10.4314/tjpr.v21i2.2
5. Wu R, Wei F, Qu L, Bai L, Li J, Li F, Yan W, Wang Q, Wei J. Effects of Keluoxin capsule combined with losartan potassium on diabetic kidney disease: study protocol for a randomized double-blind placebo-controlled multicenter clinical trial. *Trials* 2020; 21(1): 951.
6. Yu W, Duan S, Yu Z. The effect of Bailing capsules combined with losartan to treat diabetic glomerulosclerosis and the combination's effect on blood and urine biochemistry. *Am J Transl Res* 2021; 13(6): 6873-6880.
7. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol* 2020; 18(7): e3000410.
8. Hobby GP, Karaduta O, Dusio GF, Singh M, Zybailov BL, Arthur JM. Chronic kidney disease and the gut microbiome. *Am J Physiol Renal Physiol* 2019; 316(6): F1211-F1217.
9. Girndt M. [Diagnosis and treatment of chronic kidney disease]. *Internist (Berl)* 2017; 58(3): 243-256.

10. Wanner C, Ketteler M. [Chronic Kidney Disease]. *Dtsch Med Wochenschr* 2017; 142(3): 193-196.
11. Zhong J, Yang HC, Fogo AB. A perspective on chronic kidney disease progression. *Am J Physiol Renal Physiol* 2017; 312(3): F375-F384.
12. Liu W, Wang S, Sun Q, Yang Z, Liu M, Tang H. DCLK1 promotes epithelial-mesenchymal transition via the PI3K/Akt/NF-kappaB pathway in colorectal cancer. *Int J Cancer* 2018; 142(10): 2068-2079.
13. Wang D, Jin M, Zhao X, Zhao T, Lin W, He Z, Fan M, Jin W, Zhou J, Jin L, et al. FGF1(DeltaHBS) ameliorates chronic kidney disease via PI3K/AKT mediated suppression of oxidative stress and inflammation. *Cell Death Dis* 2019; 10(6): 464.
14. Sun X, Chen L, He Z. PI3K/Akt-Nrf2 and Anti-Inflammation Effect of Macrolides in Chronic Obstructive Pulmonary Disease. *Curr Drug Metab* 2019; 20(4): 301-304.
15. Garcia TE, Arellano BA, Sanchez RO, Garcia AF, Arguello GR, Loredo MM, Tapia E, Sanchez LL, Osorio AH. The Beneficial Effects of Allicin in Chronic Kidney Disease Are Comparable to Losartan. *Int J Mol Sci* 2017; 18(9).
16. He YL, Wen JG, Pu QS, Wen YB, Zhai RQ, Chen Y, Ma Y, Liu EP, Xing D, Ji FP, et al. Losartan prevents bladder fibrosis and protects renal function in rat with neurogenic paralysis bladder. *NeuroUrol Urodyn* 2021; 40(1): 137-146.
17. Pilo P, Frey J. Pathogenicity, population genetics and dissemination of *Bacillus anthracis*. *Infect Genet Evol* 2018; 64: 115-125.
18. Pflug KM, Sitcheran R. Targeting NF-kappaB-Inducing Kinase (NIK) in Immunity, Inflammation, and Cancer. *Int J Mol Sci* 2020; 21(22): 8470.