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Apelin protects against acute renal injury by inhibiting TGF-β1

Hong Chen [a,b], Danyang Wan [c], Lin Wang [c], Anlin Peng [d], Hongdou Xiao [c], Robert B. Petersen [e,f,g], Chengyu Liu [a,b], Ling Zheng [c,*], Kun Huang [a,b,**]

[a] Tongji School of Pharmacy, Huazhong University of Science and Technology, Wuhan, China, 430030
[b] Centre for Biomedicine Research, Wuhan Institute of Biotechnology, Wuhan, China, 430074
[c] College of Life Sciences, Wuhan University, Wuhan, China, 430072
[d] Wuhan the Third Hospital, Wuhan, China, 430060
[e] Department of Pathology, Case Western Reserve University, Cleveland, OH, USA, 44106
[f] Department of Neuroscience, Case Western Reserve University, Cleveland, OH, USA, 44106
[g] Department of Neurology, Case Western Reserve University, Cleveland, OH, USA, 44106

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ABSTRACT

Renal ischemia/reperfusion (I/R) injury is the most common cause of acute kidney injury, having a high rate of mortality and no effective therapy currently available. Apelin-13, a bioactive peptide, has been shown to inhibit the early lesions of diabetic nephropathy in several mouse models by us and others. To test whether apelin-13 protects against renal I/R induced injury, male rats were exposed to renal I/R injury with or without apelin-13 treatment for 3 days. Apelin-13 treatment markedly reduced the injury-induced tubular lesions, renal cell apoptosis, and normalized the injury induced renal dysfunction. Apelin-13 treatment inhibited the injury-induced elevation of inflammatory factors and *Tgf-β1*, as well as apoptosis. Apelin-13 treatment also inhibited the injury-induced elevation of histone methylation and Kmt2d, a histone methyltransferase of H3K4me2, following renal I/R injury. Furthermore, in cultured renal mesangial and tubular cells, apelin-13 suppressed the injury-induced elevation of *Tgf-β1*, apoptosis, H3K4me2 and *Kmt2d* under the *in vitro* hypoxia/reperfusion (H/R) conditions. Consistently, over-expression of apelin significantly inhibited H/R-induced elevation of *TGF-β1*, apoptosis, H3K4me2 and *Kmt2d*. The present study therefore suggests apelin-13 may be a therapeutic candidate for treating acute kidney injury.

1. Introduction

Renal ischemia/reperfusion (I/R) injury is the most common cause of acute kidney injury (AKI) after major surgery or renal transplantation in both allograft and native kidneys [1]. AKI affects 5% of all hospitalized patients with an unacceptably high rate of mortality [2]. AKI always implies a poor prognosis with no effective therapy currently available [3]. Renal I/R injury usually causes primary tubular epithelial cell injury, including tubular obstruction and reduced tubular re-absorption of NaCl. However, several studies reported that hemodynamic changes induced by renal I/R injury also occur, which lead to mesangial contraction and cessation of glomerular filtration. Thus, the interaction of microvascular (including glomerular and medullary parts) and tubular events both contribute to the pathogenesis of acute ischemic renal failure [4,5]. Although renal I/R injury has been extensively studied, the pathophysiologic process involving inflammation and cell apoptosis, as well as its relationship to the subsequent renal injury, remain to be fully elucidated.

TGF-β1 (transforming growth factor-β1), a key member of TGF super-family, has been established as the central mediator of renal fibrosis and inflammation associated with multiple progressive kidney diseases [6,7]. Fibrosis represents the final step of renal injury which ultimately leads to end-stage kidney failure [8], therefore, blocking TGF-β1 is an attractive approach to prevent renal injury.

Histone modifications, including acetylation, methylation and phosphorylation, play important roles in the regulation of transcriptional activity [9–12]. Histone acetylation, which is mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), regulates chromatin structure and controls genes expression [13]. Recent studies have shown that changes in the levels of HDACs are associated with renal I/R injury [14,15]. In addition, histone methyltransferases

Abbreviations: AKI, Acute kidney injury; ATN, Acute tubular necrosis; TGF-β1, Transforming growth factor β1; HAT, Histone acetyltransferase; HDAC, Histone deacetylase; HMTs, Histone methyltransferases; HDMs, Histone demethyltransferases; H/R, Hypoxia/reperfusion; I/R, Ischemia/reperfusion; ICAM-1, Intercellular adhesion molecule 1; PARP-1, Poly(ADP-ribose) polymerase-1; MCP-1, Monocyte chemotactic protein 1; 2K1C, Two-kidney-one-clip

* Corresponding author.
** Correspondence to: K. Huang, Tongji School of Pharmacy, Huazhong University of Science and Technology, Wuhan, China, 430030.
E-mail addresses: lzheng217@hotmail.com (L. Zheng), kunhuang2008@hotmail.com (K. Huang).

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(HMTs) and histone demethyltransferases (HDMs) play critical roles in chromatin remodeling and gene expression [16]. However, the roles of histone methylation or enzymes that regulate histone methylation in the pathogenesis of acute renal I/R injury remain unknown.

The apelin family of adipokines is derived from a 77-residue pre-proprotein with lengths varying from 12 to 36 residues [17,18]. Apelin-13, the most active member of the apelin group, has multiple biological functions including regulation of glucose balance, blood pressure, cardiovascular functions, blood vessel integrity and renal function [19–24]. APJ (angiotensin I receptor-related protein J receptor), which belongs to GPCR (G-protein-coupled receptor) family, is the only endogenous receptor for apelin. Apelin and APJ are widely expressed in a variety of tissues like kidney, adipose, brain and lung [25,26]. Several animal models indicate that the apelin–APJ system plays a critical role in the regulation of cardiovascular fluid homeostasis [27,28]. The apelinergic system (apelin and its receptor APJ) has also been suggested to be a promising therapeutic target in obesity-associated insulin resistance [29].

Recently, we and others demonstrated that administration of apelin-13 reduces kidney and glomerular hypertrophy as well as renal inflammation in type 1 diabetic mice [24,30], which makes apelin-13 a good candidate for treating diabetic nephropathy [31]. Based on this finding, we wondered whether apelin-13 might also have beneficial effects on acute renal injury, such as renal I/R injury. In the present study, we administered apelin-13 to renal I/R injured rats. The injury-induced tubular lesions, renal dysfunction, renal cell apoptosis, alterations in histone methylation and histone methyltransferase/demethylase, as well as the levels of *Tgf-β1* were analyzed. In addition, *in vitro* hypoxia/reperfusion (H/R) injury was performed on cultured renal mesangial cells and tubular cells, and the effects of administering apelin-13 and apelin over-expression on the injury-induced apoptosis, elevation of *Tgf-β1*, alterations in histone methylation and HMTs/HDMs were assessed. Apelin-13 was found to suppress renal inflammation and apoptosis in both I/R or H/R injury by inhibiting *Tgf-β1*. These results suggest that apelin-13 should be considered as a new therapeutic target for the treatment of AKI.

2. Materials and methods

2.1. Animals and renal ischemia/reperfusion (I/R) model

Male Wistar rats were obtained from the Hubei Animal Laboratory, and housed in ventilated microisolator cages with free access to water and food. Rats weighing 180 ± 20 g were used and assigned to one of the following groups: CT group, uninjured rats with vehicle administration; I/R group, rats underwent I/R injury with vehicle administration; I/R + Ap group, rats underwent I/R injury with 5 μg/kg BW apelin-13 administered twice per day. Apelin-13 was chemically synthesized by standard Fmoc strategy and purified to >98% with rp-HPLC as we previously described [32,33]. I/R injury was performed as previously described [34]. Briefly, rats were anesthetized and underwent midline abdominal incisions with their left renal pedicle bluntly clamped by a clamp for 30 min (unilateral renal occlusion). After removing the clamps, wounds were sutured and the animals were allowed to recover for 3 days before sacrifice. Control animals were sham operated. All animal experiments were approved by the Committee on Ethics in the Care and Use of Laboratory Animals of the College of Life Sciences, Wuhan University.

2.2. Assessment of renal function

Twenty-four-hour urine samples were collected in metabolic cages 1 day before sacrifice. Urine levels of creatinine and total protein were measured with an Olympus AU2700 automatic biochemistry analyzer using a creatinine reagent kit (Fuxing Changzheng Medical Inc., Shanghai, China) or a total protein reagent kit (Greatwall Clinical Reagent Inc., Baoding, China) as we previously described [24].

2.3. Renal histology

Renal sections were stained with hematoxylin and eosin (H&E). Hallmarks of acute tubular necrosis (ATN) were examined on a double-blind basis. The degree of renal damage was semi-quantitatively evaluated with a scale in which 0 represents no abnormalities, and 1+, 2+, 3+, 4+ stand for slight (up to 20%), moderate (20 to 40%), severe (40 to 60%), and total necrosis (affecting more than 80% of renal parenchyma), respectively [35].

2.4. TUNEL assay

Paraffin-embedded sections were deparaffinized and rehydrated as previously reported [24]. Apoptotic cells were detected by TUNEL assay using an In Situ Cell Death Detection Kit (Roche, Mannheim, Germany) as previously described [36]. At least six different areas per renal sample of TUNEL positive cells were counted using an Olympus BX60 microscope equipped with a digital CCD and reported as number of TUNEL-positive nuclei divided by the total number of cells per field.

2.5. Cell culture and in vitro H/R injury

HBZY-1 (a rat mesangial cell line) and NRK-52E (a rat tubular epithelia cell line) were cultured in DMEM media (Hyclone, South Logan, USA) with 5.5 mM glucose plus 5% fetal bovine serum (FBS) (Gibco, Grand Island, USA), and maintained in a 37 °C incubator (Thermo scientific, Marietta, USA) with humidified atmosphere of 21% O_2 which was regarded as the normal culture condition. *In vitro* H/R experiments were performed as previously described with slight modifications [37]. At 80% confluence, cells were put into a 37 °C incubator under 1% O_2 with the normal media replaced with media lacking glucose and FBS, which was regarded as hypoxia. After culturing under the hypoxia condition for a specified amount of time (4 hrs for HBZY-1 cells and 1 hr for NRK-52E cells), cells were returned to the normal culture condition for two hours, which was regarded as reperfusion. For apelin-13 treatment, 300 pM apelin-13 was added to media during the hypoxia and reperfusion period as in our previous report [24]. 2-TCP (*trans*-2-Phenylcyclopropylamine hydrochloride), a HMT inhibitor [38], was added to media to induce the total methylation of H3K4 in the cultured renal cells.

2.6. Transfection

HBZY-1 cells were plated in six-well plates and transfected the next day with either pPCAGSIH-β-gal or pPCAGSIH-apelin plasmids (kind gifts from Dr. Takakura, Osaka University) using Fugene HD transfection reagent (Promega, Madison, USA) according to the manufacturer's instruction.

2.7. Western blots

Freshly collected kidney or cultured cells were sonicated in ice-cold RIPA buffer (Beyotime, Haimen, China) and protein concentrations were quantitated as previously described [39]. 20–80 μg of protein from each sample were separated by SDS-PAGE. The proteins were transferred onto PVDF membranes for immunodetection. The list of antibodies used in the present study is provided in Supplementary Table S1. The intensity of the targeted protein bands was evaluated using Quantity One 1-D Analysis Software. Individual protein levels were quantitated relative to the β-actin level in the same sample and further normalized to the respective control group, which was set at one.

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