Spironolactone rescues renal dysfunction in obstructive jaundice rats by upregulating ACE2 expression

Er-liang Kong¹ · Jin-min Zhang¹ · Ni An¹ · Yong Tao¹ · Wei-feng Yu¹ · Fei-xiang Wu¹

Received: 14 February 2018 / Accepted: 17 April 2018 / Published online: 7 June 2018
© The International CCN Society 2018

Abstract

Postoperative acute renal failure in patients with obstructive jaundice is still a serious clinically complication, yet the mechanisms remain unclear. Renin-angiotensin-aldosterone system (RAAS) plays a central role in renal disease progression. Several lines of evidence shows that angiotensin-converting-enzyme-2 (ACE2), a main effector of RAAS acts as a local regulator for renal protection. This study aims to investigate the role of ACE2 and the effect of spironolactone treatment in obstructive jaundice(OJ) rats with renal injury. The rats with obstructive jaundice were established by bile duct ligation. Total bilirubin (TBil), serum creatinine (Scr) and the expression of ACE2 in kidney tissue of obstructive jaundice rats were detected. Comparatively, the expression of ACE2, renin, angiotensin II (AngII), angiotensin-(1–7)[Ang-(1–7)], aldosterone and intercellular adhesion molecule 1 (ICAM-1) in kidney tissues after spironolactone administration were measured by ELISA. Renal necrosis, inflammation and fibrosis induced by OJ were also measured by HE staining and Masson staining. The correlation between the expression of ACE2 and TBil, also the Scr level were investigated. With the time of common bile duct ligation prolonged, the TBil and Scr concentration increased while the expression of ACE2 in OJ rats' kidney tissues decreased. However, after spironolactone intervention, the expressions of ACE2, renin, AngII, Ang-(1–7), aldosterone and ICAM-1 in kidney tissue were changed, moreover, necrotic, inflammatory and fibrotic condition was also decreased. The relationship between the mRNA expression of ACE2 and TBil/Scr was observed to be moderately negatively correlated ($r = -0.516$, $R^2 = 0.292$, $P < 0.01$), ($r = -0.576$, $R^2 = 0.332$, $P < 0.01$), respectively. RAAS exerted an important effect in the renal damage caused by OJ. Spironolactone intervention not only improved the degree of renal fibrosis induced by OJ, but also upregulated the ACE2 expression in the kidney of OJ rats and rescued the renal function.

Keywords Obstructive jaundice (OJ) · Renal function · Angiotensin-converting-enzyme-2 (ACE2) · Renin-angiotensin-aldosterone system (RAAS) · Spironolactone

Abbreviations

OJ	Obstructive jaundice
RAAS	Renin-angiotensin-aldosterone system
TBil	Total bilirubin
Scr	Serum creatinine

ACE2	Angiotensin-converting-enzyme-2
AngII	Angiotensin II
[Ang-(1–7)]	Angiotensin-(1–7)
ICAM-1	Intercellular adhesion molecule 1
RAS	Renin angiotensin system
SO group	Sham-operated group
BDL group	Bile duct ligation group
HE	Hematoxylin and eosin
BDL + S group	Bile duct ligation with spironolactone treatment group

Er-liang Kong, Jin-min Zhang and Ni An contributed equally to this work.

✉ Wei-feng Yu
ywf808@sohu.com

✉ Fei-xiang Wu
feixiangwu@hotmail.com

¹ Department of Anesthesiology & Intensive Care, Shanghai Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai 200438, China

Background

Postoperative acute renal failure in patients with obstructive jaundice (OJ) has been originally observed by

surgeons more than a century ago and led to focus on the pathological mechanisms and therapies protect against the complication in patients with obstructive jaundice (Richard 1989). Acute renal failure occurs in 8 to 10% of patients requiring surgery for relief of obstructive jaundice and contributes to eventual mortality in 70 to 80% of those who develop it (al Makdessi et al. 1995). Accumulating clinical and biochemical evidence supports the notion that the strongly rise of Tbi and Scr level in postoperative patients with OJ contribute to the acute renal failure (Krieger and Kimmig 1995).

Given that information, appropriate nursing intervention in postoperative for patients with OJ play an important role in decreasing the rate of organ dysfunction syndrome like acute renal failure, which are life threatening complication. Present studies have not clearly clarified the mechanism of renal failure caused by surgery in patients with OJ. However, studies on pathobiology of renal disease show that RAAS has a significant influence on renal disease progression. Blockade of the RAAS by renin inhibition and aldosterone blockade is the effective way to prevent progressive renal dysfunction in chronic renal diseases. Yet in patients with renal diseases, blockade of the RAAS shows incomplete renoprotective effect but at high risk of renal risk. Thus, the exact role of RAAS in the renal damage caused by OJ is still unclear.

Previous research mainly focused on the changes of angiotensin II (AngII) and aldosterone levels, as well as the effect of angiotensin receptor blocker (ARB) and angiotensin-converting enzyme inhibitor (ACEI) on renal function. However, these researches reported inconsistent results due to the differences in observe duration and experimental animal species (Wistar or Sprague-Dawley rats), etc., when the changes of serum RAAS components were tested during OJ (O'Neill et al. 1990; Poo et al. 1997; Ubada et al. 1994).

Until 2000, ACE2 was discovered to have a significant impact on RAAS and was demonstrated to be a therapeutic target in kidney disease[5]. Angiotensin-converting-enzyme-2 (ACE2) is also known as an important protective factor of cardiac, cerebral and renal function during vascular diseases, such as diabetes and hypertension. Through balancing the expression of ACE2 and increasing the decomposition of AngII to angiotensin-(1-7) [Ang-(1-7)], ACE2 is observed an important regulatory role in RAAS in both diabetic patients and animal models, and its upregulation results in a significant protective effect on renal function (Clarke and Turner 2012).

Aldosterone participates in the formation of organ fibrosis. Spironolactone, as an aldosterone receptor antagonist (Trachtman et al. 2004), can reduce the collagen synthesis in kidney, etc. Another research showed that activation of the local RAAS in the liver was closely related to the formation of liver fibrosis (Caligiuri et al. 2003).

Animal experiments showed that both the increase of concentration and duration of aldosterone administration could reduce the expression of ACE2 and even increase cell apoptosis, and aldosterone receptor antagonists could reverse the above pathological changes (Yamamuro et al. 2008).

In the present study, we investigated the role of ACE2 in OJ rats with renal injury and spironolactone intervention is effective in rescuing renal injury caused by OJ.

Methods

Ethics statement

All the animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the Institutional Animal Care and Use Committee of Second Military Medical University, and the Guide for the Care and Use of Laboratory Animals according to the regulation in the People's Republic of China. All animals were provided by Shanghai Experimental Animal Center, P. R. China. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Animals

Adult male Sprague-Dawley (SD) rats (weighing 180–220 g) were obtained from the Shanghai Slac Experimental Animal Centre (Shanghai, China). All animals received humane care, and the study protocol was approved by the Second Military Medical University Animal Care Committee. The rats were housed in individual cages in a temperature-controlled room with alternating 12 h light/dark cycles. Food was withheld 8 h before the start of experiments, whereas all animals had free access to water. Monitoring for health problems was performed three times a day and no death was found during the experiments. All rats were sacrificed after collecting blood and kidney specimens after the experiments.

Establishment of OJ model

The OJ model was established by bile duct ligation (BDL). Rats were injected chloral hydrate into abdomen after weighing (300 mg/kg), abdomen were opened and bile ducts separated, the main bile ducts were ligated using two ligatures approximately 0.5 cm apart and then transected at the midpoint between the two ligatures. Bile ducts were isolated without ligation in the sham rats after opening the abdomen. Cholestasis was confirmed by increased serum level of TBil as well as intact bile duct

ligature and proximal dilation of the bile duct at the time of sacrifice (Kaler et al. 2004; Pereira et al. 2008; Tao et al. 2014).

Aldosterone antagonist spironolactone treatment

Rats were divided into three groups: Sham-operated (SO) group ($n = 14$), BDL group ($n = 14$) and BDL + S group (spironolactone $40 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, $n = 14$). The BDL + S group was orally given spironolactone (SINE, Shanghai, China) daily for 4 days or 11 days with the first administration starting on day 3 after surgery. The SO group and BDL group underwent administration of the same volume of sterile saline solution on the same days. All rats were sacrificed after collecting blood and kidney specimens.

Measurement of plasma levels of TBil and Scr

Plasma levels of TBil and Scr were determined by using an automated biochemistry analyzer (Olympus, Tokyo, Japan).

Histopathological examination

Formalin-fixed kidney specimens were embedded in paraffin, and stained with hematoxylin and eosin (HE) and Masson according to the manufacturer's instructions. The staining images were acquired using a light microscope (Olympus BX51, Tokyo, Japan) with $200\times$ magnification.

Measurement of ACE2 mRNA expression

Quantitative real-time Polymerase Chain Reaction (PCR) was used to confirm and validate the expression of ACE2 in kidney tissue. Total RNA was extracted from the kidney tissue of the rats using Trizol (Invitrogen, Grand Island, NY, USA) according to the manufacturer's guidelines. The total RNA was reverse transcribed using dNTPs and M-MLV Reverse Transcriptase (promega). Real-time PCR was performed using SYBR Master Mixture PCR kits (TAKARA Biotechnology, Dalian, China) and ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Primers were designed by Rothen Pharma Co (Shanghai, China) (Table 1). The expression level of the target gene was normalized to the GAPDH gene expression. Data were analyzed by ABI Prism 7500 SDS Software.

Analysis of ACE2, renin, AngII, Ang-(1–7), aldosterone and ICAM-1 in kidney tissue after spironolactone administration by ELISA

The tissue preparation for ELISA is described as following. Rinse tissue with PBS to remove excess blood, chopped into 1–2 mm pieces, and homogenize with a

Table 1 Primer Sequences for Quantitative real-time Polymerase Chain Reaction

Target gene	Sequence number	Primer sequence
ACE2	NM_001012006	F: GGTCTTCTGCCATCCAATT R: ACCTCCACTTCTCTAACAT
GAPDH	NM_017008	F: TCCCTCAAGATTGTCAGCAA R: AGATCCACAACGGATACATT

tissue homogenizer in PBS or in a tissue lysate solution. Centrifuge at approximately $5000 \times g$ for 5 min. Assay immediately or aliquot and store homogenates at -20°C . Avoid repeated freeze-thaw cycles. The samples were analyzed using commercially available enzyme-linked immunosorbent assays (ELISA) according to the manufacturers' instructions. ACE2 was measured using the Rat ACE2 ELISA Kit (Elabscience, Wuhan, China). Renin was measured using the Rat REN ELISA Kit (Elabscience, Wuhan, China). Angiotensin II (AngII) was measured using the Mouse Ang-II ELISA Kit (Elabscience, Wuhan, China). Ang-(1–7) was measured using the Rat Ang-(1–7) ELISA Kit (Lanpai BIO, Shanghai, China). Aldosterone was measured using the Rat ALD ELISA Kit (Elabscience, Wuhan, China). ICAM-1 was measured using the Rat sICAM-1/CD54 ELISA Kit (Elabscience, Wuhan, China).

Immunohistochemical evaluation of ACE2 in kidney

The expression level of ACE2 in the kidney tissue was examined by immunohistochemistry. Immunohistochemical staining for ACE2 antibody (1:200, Abcam, Cambridge, UK) was performed using the avidin–biotin–peroxidase complex method. All slides were observed and photographed under a light microscope (Olympus BX51, Tokyo, Japan).

Data analysis

All data was expressed as mean $\pm$ standard deviation and analyzed by PASW Statistics 18 statistical software. Statistical analysis was performed using one-way or two-way ANOVA. Results were considered statistically significant when $P < 0.05$. The Pearson correlation coefficient was interpreted using the scale provided by Salkin, where r between 0.8 and 1.0 (or -0.8 and -1.0) is defined as very strong, between 0.6 and 0.8 as strong, between 0.4 and 0.6 as moderate, between 0.2 and 0.4 as weak and between 0.0 and 0.2 as very weak or no relationship (Salkind and Rasmussen 2007).

Data Availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Results

BDL induces renal dysfunction

Scr and TBil increased in BDL rats As shown in Table 2, Serum concentrations of TBil was significantly higher in the BDL group compared with that of the SO group ($P < 0.01$) on day 3, 7, 10 and 14 after surgery (Fig. 1a). Scr was significantly higher in the BDL group compared with that of the SO group ($P < 0.01$) on day 7, 10 and 14 after surgery (Fig. 1b).

ACE2 expression in kidney was upregulated in BDL rats The ACE2 mRNA in kidney of BDL and SO rats was detected with Quantitative real-time PCR method. As shown in Fig. 2f, ACE2 mRNA decreased significantly in the BDL group compared with that of the SO group on day 14 after surgery ($P < 0.05$), while there was no significant change between two groups on day 3 ($p = 0.84$), day 7 ($p = 0.27$) and day 10 ($p = 0.06$). These results suggested that with the time of common bile duct ligation prolonged, the TBil concentration increased, meanwhile the expression of ACE2 in kidney tissue decreased.

As shown in Fig. 2a-e, the renal tubules were the main expression areas of ACE2 and fewer ACE2 antibody-positive areas (tan areas) were observed in BDL group on day 7, 10 and 14 after surgery compared with those of the SO group. And there was no significant change between BDL group and SO group on day 3.

ACE2 expression is negative correlated to TBil/Scr in BDL rats The relationships between the mRNA expressions of ACE2 and TBil/Scr were shown in Figs. 3a and b, respectively. The correlation between the mRNA expressions of ACE2 and TBil was tested using the linear correlation coefficients, which was observed to be moderately negatively correlated ($r = -0.516$, $R^2 = 0.292$, $P < 0.01$) (Fig. 3a). The correlation between the mRNA expressions of ACE2 and Scr was observed to be moderately negatively correlated ($r = -0.576$, $R^2 = 0.332$, $P < 0.01$) (Fig. 3b).

Spironolactone improves renal function induced by BDL

Scr was decreased after spironolactone administration in BDL rats As shown in Fig. 1c, Scr was significantly higher in the BDL group compared with that of the SO group on day 7 after surgery ($P < 0.05$). After administration of spironolactone ($40 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) for 4 consecutive days, the BDL + S group showed reduced Scr level compared with those in the BDL group ($P < 0.05$), whereas the comparison between the SO group and the BDL + S group showed no statistical significant difference ($P = 0.120$). Scr was significantly higher in the BDL group

Table 2 Serum levels of TBil and serum Scr on day 3, 7, 10 and 14 after surgery in BDL and SO group

	TBil ($\mu\text{mol/L}$)		Scr ($\mu\text{mol/L}$)	
	SO Group	BDL Group	SO Group	BDL Group
3 day	6.95 ± 0.25	$80.99 \pm 8.25^*$	36.56 ± 6.45	46.79 ± 2.09
7 day	7.03 ± 0.63	$123.83 \pm 13.96^*$	37.61 ± 5.80	$50.71 \pm 1.09^\#$
10 day	6.69 ± 0.84	$114.27 \pm 8.11^*$	36.64 ± 6.60	$57.29 \pm 3.31^\#$
14 day	6.77 ± 0.87	$142.54 \pm 24.24^*$	37.56 ± 6.18	$71.41 \pm 21.16^\#$

Serum concentration of TBil was significantly higher in the BDL group compared with the SO group ($*P < 0.05$) on day 3, 7, 10 and 14 after surgery. Scr was significantly higher in the BDL group compared with the SO group on day 7, 10 and 14 after surgery ($^\#P < 0.05$). Total Bilirubin was indicated as TBil, serum creatinine was indicated as Scr, Bile Duct Ligation group was indicated as BDL group, and Sham-operated group was indicated as SO group

compared with that of the SO group on day 14 after surgery ($P < 0.05$). After administration of spironolactone ($40 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) for 11 consecutive days, the BDL + S group showed increased Scr level compared with those in the SO group ($P < 0.05$), whereas the comparison between the BDL group and the BDL + S group showed no statistical significant difference ($P = 0.071$). The study showed that with the time of obstruction prolonged, the concentration of Scr increased, which could be decreased by spironolactone intervention.

The decrease of ACE2 was reversed in BDL rats after spironolactone administration As shown in Table 3, the ACE2 expression in kidney was significantly lower in the BDL group compared with that of the SO group on day 7 and day 14 after surgery ($P < 0.05$), and was significantly lower in the BDL + S group compared with that of the SO group on day 14 after surgery ($P < 0.05$). The ACE2 expression in kidney was significantly higher in the BDL + S group compared with that of the BDL group on day 7 and day 14 after surgery ($P < 0.05$). The study showed that with the time of obstruction prolonged, the expression of ACE2 reduced in the kidney, which could be reversed by spironolactone intervention.

The increase of ICAM-1 expression was slowed down in BDL rats after spironolactone administration As shown in Fig. 1d, the ICAM-1 expression in kidney was significantly higher in the BDL group and BDL + S group compared with that of the SO group on day 7 and day 14 after surgery ($P < 0.05$), and was significantly lower in the BDL + S group compared with the BDL group on day 7 and day 14 after surgery ($P < 0.05$). The study showed that with the time of obstruction prolonged, the expression of ICAM-1 upregulated in the kidney, which could be prevented by spironolactone intervention.

Fig. 1 BDL induces renal dysfunction. **a**, TBil increased in BDL rats compared to the SO rats. **b**, Scr increased in BDL rats compared to the SO rats. **c**, Scr was decreased after spironolactone administration. **d**, ICAM-1 expression decreased after spironolactone administration. Total Bilirubin levels and Scr levels in different groups was examined by using an

automated biochemistry analyzer. The ICAM-1 expression was examined by ELISA. SO group: Sham-operated group; BDL group: Bile Duct Ligation group; * $P < 0.05$ vs. SO group, # $P < 0.05$ vs. BDL group ($n = 7$ for each group at each time point)

Renin was decreased after spironolactone administration As shown in Table 3, the renin expression in kidney was significantly higher in the BDL group and BDL + S group compared with that of the SO group on day 7 and day 14 after surgery ($P < 0.05$), and was significantly lower in the BDL + S group compared with that of the BDL group on day 14 after surgery ($P < 0.05$), whereas the comparison between the BDL group and the BDL + S group showed no statistical significant difference on day 7 ($P = 0.665$). The study showed that with the time of obstruction prolonged, the expression of renin increased in the kidney, which could be prevented by spironolactone intervention.

AngII was decreased after spironolactone administration As shown in Table 3, the AngII expression of kidney was significantly higher in the BDL group and BDL + S group compared with that of the SO group on day 7 and day 14 after surgery ($P < 0.05$), and was significantly lower in the BDL + S group compared with that of the BDL group on day 14 after surgery ($P < 0.05$), whereas the comparison between the BDL group and the BDL + S group showed no statistical significant difference on day 7 ($P = 0.151$). The study showed that with the time of obstruction prolonged, the expression of AngII upregulated in the kidney, which could be prevented by spironolactone intervention.

Ang-(1–7) was increased after spironolactone administration As shown in Table 3, the Ang-(1–7) expression in kidney was significantly lower in the BDL group and BDL + S group compared with that of the SO group on day 7 and day 14 after surgery ($P < 0.05$), and was significantly higher in the BDL + S group compared with that of the BDL group on day 7 and day 14 after surgery ($P < 0.05$). The study showed that with the time of obstruction prolonged, the expression of Ang-(1–7) reduced in the kidney, which could be prevented by spironolactone intervention.

Aldosterone was decreased after spironolactone administration As shown in Table 3, the aldosterone expression in kidney was significantly higher in the BDL group and BDL + S group compared with that of the SO group on day 7 and day 14 after surgery ($P < 0.05$), and was significantly lower in the BDL + S group compared with that of the BDL group on day 7 and day 14 after surgery ($P < 0.05$). The study showed that with the time of obstruction prolonged, the expression of aldosterone upregulated in the kidney, which could be prevented by spironolactone intervention.

HE staining and Masson staining of the kidney tissue As shown in Fig. 4, HE staining results indicated that the kidney tissue of rats in the BDL group presented obviously increased

Fig. 2 BDL downregulates ACE2. **a-e**, ACE2 expression levels in different groups were examined by immunohistochemistry in renal tubules; ACE2 positive areas (tan areas) were observed in BDL group on day 7(C), 10(D) and 14(E) compared with SO group (A). No significant change between BDL group on day 3 (B). **a1-e1**, The

magnified views of A-E. **f**, ACE2 mRNA levels in different groups were tested by RT-PCR. ACE2 expression in kidney was decreased in BDL rats; SO group: Sham-operated group; BDL group: Bile Duct Ligation group; * $P < 0.05$ vs. SO group ($n = 7$ for each group at each time point)

areas of necrotic and inflammatory cells infiltration compared with that of the SO group, and was lower in the BDL + S group compared with that of the BDL group after surgery. In Fig. 5, Masson staining results indicated that the kidney tissue of rats in the BDL group presented increased areas of interstitial fibers compared with that of the SO group, and was lower in the BDL + S group compared with that of the BDL group after surgery.

Discussion

Organ dysfunction syndrome like renal failure was more easily occurred in patients with OJ during perioperative period than those without OJ (Pain et al. 1985; Wait and Kahng 1989). The histopathology changes occurred in human kidney tissue which can be observed even in short-term biliary obstruction (Uslu et al. 2010). Padillo's research found that the renal dysfunction in patients with OJ was related to derangements in plasma levels of aldosterone and renin, etc. (Padillo et al. 2005).

In 2000, two foreign research groups firstly cloned a homologue of human ACE from the human cDNA library of lymphoma and left ventricular tissue of heart failure, respectively, and named it angiotensin-converting enzyme homologue (ACE2 or ACEH) (Iwai and Horiuchi 2009; Tipnis et al. 2000). The physiological and pathophysiological roles of ACE2 have been characterized in the lung, kidney, and cardiovascular system (Hamming et al. 2007). ACE2 is an important enzyme in the production of Ang-(1-7) which has a close relationship with the G protein-coupled receptor Mas. The ACE2- Ang-(1-7)-Mas pathway can battle with the effects of the conventional ACE-AngIIaxis, and Ang-(1-7) can balance the effects of AngII on organs such as the kidney (Ferrario et al. 2005). Studies have shown that ACE2 is not only an important cardiac function regulator, but also a direct protective factor of renal function (Donoghue et al. 2003; Soler et al. 2008). ACE2 can efficiently convert AngII to Ang-(1-7), thereby stimulating Mas, a G protein-coupled receptor (Pinheiro and Simoes 2012; Raizada and Ferreira 2007). Through balancing the expression of ACE2 and increasing the decomposition of AngII to Ang-(1-7), ACE2 is

Fig. 3 ACE2 have negative correlation between TBil/Scr. **a**, ACE2 mRNA expression is negative correlated to TBil. The correlation between ACE2 mRNA expression and TBil was observed to be moderately negatively correlated ($r = -0.516$, $R^2 = 0.292$, $P < 0.01$). **b**, ACE2 mRNA expression is negative correlated to Scr. The correlation between ACE2 mRNA expression and Scr was observed to be moderately negatively correlated ($r = -0.576$, $R^2 = 0.332$, $P < 0.01$)

considered as an important regulatory role in RAAS in both diabetic patients and animal models, its upregulation results in a significant protective effect on renal function (Wakahara et al. 2007). Studies showed that the administration of ACE2 agonist have a protective effect on pulmonary hypertension, ischemic stroke and hypertensive cardiac dysfunction (Mecca et al. 2011). As it was shown in some research, the high ACE/

ACE2 ratio in kidneys of patients with type 2 diabetes with overt nephropathy may contribute to renal injury.

Aldosterone is a steroid hormone which influence blood volume and blood pressure by acting on endothelial tissue to retain sodium and discharge potassium, and, the same as other RAAS components, it also has an independent local system within the kidney. The role of aldosterone is far more than simply affecting fluid metabolism as recognized before, it can also induce vascular damage and fibrosis of the kidney. Some studies showed that ACE2 in the kidney was down-regulated by aldosterone, which induced acute kidney injury (Fukuda et al. 2011). This is the reason why our study aimed to spironolactone for clinical drug intervention.

Spironolactone, a non-selective aldosterone antagonist, is commonly used in clinical practice and is a kind of drug that interferes with RAAS. Some researchers showed spironolactone treatment in patients after decompression of the biliary tract can reduced postoperative mortality (Riabtsev and Solomka Ia 1994). Another researcher showed that the activation of local RAAS in the liver was closely related to the formation of liver fibrosis (Caligiuri et al. 2003). Reports on tissue injury by aldosterone showed that inflammation played important roles in the remodeling of the kidney, etc. (Cha et al. 2005; Gilbert and Brown 2010) Hostetter et al. proved that the aldosterone in the blood circulation would indirectly promote the development of chronic cardiac, cerebral and renal diseases by inducing inflammation, fibrosis and necrosis (Hostetter et al. 2001).

In our study, we found that with the time of common bile duct ligation prolonged, not only the TBil concentration increased, but also Scr level gradually increased, which means that BDL induced renal dysfunction was accompanied with the change of Scr/TBil. Meanwhile, the down regulation of the ACE2 expression in the kidney of the BDL group was significantly different from that of the SO group. Interestingly, ACE2 expression is

Table 3 ELISA analysis of ACE2, renin, AngII, Ang-(1–7) and ALD in kidney tissue after spironolactone administration

Group	ACE2 (ng/ml)	Renin (pg/ml)	Ang II (pg/ml)	Ang-(1–7) (pg/ml)	Aldosterone (pg/ml)
SO group(7d)	6.46 ± 0.64	428.10 ± 45.46	407.05 ± 22.33	106.90 ± 14.29	128.73 ± 15.12
BDL group(7d)	5.12 ± 0.45*	487.21 ± 49.78*	527.43 ± 100.04*	77.22 ± 12.22*	232.30 ± 21.51*
BDL + S group(7d)	6.01 ± 0.53	477.45 ± 32.81*	484.46 ± 31.58*	91.29 ± 11.55*#	173.96 ± 21.81*#
SO group(14d)	6.57 ± 0.36	434.06 ± 38.71	387.68 ± 21.20	107.70 ± 12.32	125.28 ± 14.76
BDL group(14d)	3.53 ± 0.20*	745.25 ± 41.85*	710.64 ± 67.96*	34.09 ± 9.25*	384.57 ± 20.18*
BDL + S group(14d)	4.54 ± 0.41*#	572.77 ± 40.18*#	601.48 ± 38.39*#	49.78 ± 10.68*#	297.30 ± 24.64*#

* $P < 0.05$ compared with SO group on the same day, # $P < 0.05$ compared with BDL group on the same day, Enzyme-linked immunosorbent assay was indicated as ELISA, Angiotensin-converting-enzyme-2 was indicated as ACE2, AngiotensinII was indicated as AngII, Angiotensin 1–7 was indicated as Ang-(1–7), Bile Duct Ligation group was indicated as BDL group, Sham-operated group was indicated as SO group, Bile Duct Ligation rats with Spironolactone Treatment group was indicated as BDL + S group

Fig. 4 Spironolactone have renoprotective effect on the kidney tissue of BDL rats. By HE staining, Areas of necrotic and inflammatory cell infiltration (the black arrow) in BDL group on day 7 (b), 14 (d) and BDL + S group on day 7 (c), 14 (e) after surgery compared with SO

group (a). HE: Hematoxylin and eosin staining; SO group: Sham-operated group; BDL group: Bile Duct Ligation group; BDL + S group: Spironolactone Treatment group

negatively correlated to Scr/Tbil. As ACE2 was considered as a renoprotective factor (Moon et al. 2008; Reich et al. 2008), we hypothesized that the downregulation of ACE2 would weaken the protective effect, thereby leading to the worsening of renal damage. Further investigation was shown that that spironolactone intervention could significantly improve the degree of renal fibrosis induced by OJ as shown in Masson staining and downregulate the expression of ICAM-1 as proved by ELISA. Speculations are that spironolactone may conduct its renal protective effects through alleviating inflammatory responses and fibrosis formation. Although in some previous study, renal alterations are only functional rather the morphological in

BDL models, still some other studies have shown that transport and nonspecific morphological changes in the proximal convoluted tubule occur 3 to 4 days following bile duct ligation in rats (Kaler et al. 2004). We also discovered that with the increase of TBil and Scr, the expressions of ACE2 and Ang-(1–7) reduced in the kidney, whereas the expressions of renin, AngII and aldosterone increased, the ACE2 expression level of BDL group could be upregulated by spironolactone intervention. It implied that administration of spironolactone might induce corresponding feedback regulation of RAAS, thereby affecting the expression of relevant factors including ACE2 and improving the renal function. This study did not discuss

Fig. 5 Spironolactone alleviates the interstitial fibrosis in BDL rats. Areas of interstitial fibrosis (the black arrow) in BDL group (b, d) and BDL + S group (c, e) compared with the SO group (a) by using Masson

staining. SO group: Sham-operated group; BDL group: Bile Duct Ligation group; BDL + S group: Spironolactone Treatment group

the mechanism that led to the expression change of ACE2 by the spironolactone.

Conclusions

In summary, in the OJ model we observed that Scr/TBil concentration increased and the ACE2 expression decreased in the kidney tissue, which proved that the change of ACE2 expression was correlated with the renal damage of OJ in rats. Spironolactone intervention not only improved the degree of renal fibrosis induced, but also upregulated ACE2 expression in the kidney of OJ.

Acknowledgements The results presented in this paper have not been published previously in whole or part.

Authors' contributions EK and NA performed the experiments and analyzed the data; EK, NA, JZ, YT, XM, WY and FW conceived and designed the experiments; WY and FW analyzed the data and EK, NA and JZ contributed the writing of the manuscript. EK and NA contributed equally to this work. All authors read and approved the final manuscript.

Compliance with ethical standards

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

References

- al Makdessi S, Brandle M, Ehrh M, Sweidan H, Jacob R (1995) Myocardial protection by ischemic preconditioning: the influence of the composition of myocardial phospholipids. *Mol Cell Biochem* 145(1):69–73
- Caligiuri, A., De Franco, R. M., Romanelli, R. G., Gentilini, A., Meucci, M., Failli, P., ... Pinzani, M. (2003). Antifibrogenic effects of canrenone, an antialdosterone drug, on human hepatic stellate cells. *Gastroenterology* 124(2):504–520. <https://doi.org/10.1053/gast.2003.50058>
- Cha, D. R., Kang, Y. S., Han, S. Y., Jee, Y. H., Han, K. H., Kim, H. K., ... Kim, Y. S. (2005). Role of aldosterone in diabetic nephropathy. *Nephrol (Carlton)* 10(Suppl): S37–39. <https://doi.org/10.1111/j.1440-1797.2005.00455.x>
- Clarke, N. E., & Turner, A. J. (2012). Angiotensin-converting enzyme 2: the first decade. *Int J Hypertens* 307315. <https://doi.org/10.1155/2012/307315>
- Donoghue, M., Wakimoto, H., Maguire, C. T., Acton, S., Hales, P., Stagliano, N., ... Breitbart, R. E. (2003). Heart block, ventricular tachycardia, and sudden death in ACE2 transgenic mice with down-regulated connexins. *J Mol Cell Cardiol* 35(9):1043–1053
- Ferrario CM, Trask AJ, Jessup JA (2005) Advances in biochemical and functional roles of angiotensin-converting enzyme 2 and angiotensin-(1-7) in regulation of cardiovascular function. *Am J Physiol Heart Circ Physiol* 289(6):H2281–H2290. <https://doi.org/10.1152/ajpheart.00618.2005>
- Fukuda S, Horimai C, Harada K, Wakamatsu T, Fukasawa H, Muto S, Itai A, Hayashi M (2011) Aldosterone-induced kidney injury is mediated by NFκB activation. *Clin Exp Nephrol* 15(1):41–49. <https://doi.org/10.1007/s10157-010-0373-1>
- Gilbert KC, Brown NJ (2010) Aldosterone and inflammation. *Curr Opin Endocrinol Diabetes Obes* 17(3):199–204
- Hamming, I., Cooper, M. E., Haagmans, B. L., Hooper, N. M., Korstanje, R., Osterhaus, A. D., ... van Goor, H. (2007). The emerging role of ACE2 in physiology and disease. *J Pathol* 212(1):1–11. <https://doi.org/10.1002/path.2162>
- Hostetter TH, Rosenberg ME, Ibrahim HN, Juknevicus I (2001) Aldosterone in renal disease. *Curr Opin Nephrol Hypertens* 10(1): 105–110
- Iwai M, Horiuchi M (2009) Devil and angel in the renin-angiotensin system: ACE-angiotensin II-AT1 receptor axis vs. ACE2-angiotensin-(1-7)-mas receptor axis. *Hypertens Res* 32(7):533–536. <https://doi.org/10.1038/hr.2009.74>
- Kaler B, Karim T, Morgan WA, Bach PH, Yousef IM, Bomzon A (2004) Are bile acids involved in the renal dysfunction of obstructive jaundice? An experimental study in bile duct ligated rats. *Ren Fail* 26(5): 507–516
- Krieger H, Kimmig P (1995) Survival ability of trichomonas vaginalis in mineral baths. *Gesundheitswesen* 57(12):812–819
- Mecca AP, Regenhardt RW, O'Connor TE, Joseph JP, Raizada MK, Katovich MJ, Summers C (2011) Cerebroprotection by angiotensin-(1-7) in endothelin-1-induced ischaemic stroke. *Exp Physiol* 96(10):1084–1096. <https://doi.org/10.1113/expphysiol.2011.058578>
- Moon JY, Jeong KH, Lee SH, Lee TW, Ihm CG, Lim SJ (2008) Renal ACE and ACE2 expression in early diabetic rats. *Nephron Exp Nephrol* 110(1):e8–e16. <https://doi.org/10.1159/000149586>
- O'Neill P, Wait RB, Kahng KU (1990) Obstructive jaundice and renal failure in the rat: the role of renal prostaglandins and the renin-angiotensin system. *Surgery* 108(2):356–362
- Padillo FJ, Cruz A, Briceno J, Martin-Malo A, Pera-Madrado C, Sitges-Serra A (2005) Multivariate analysis of factors associated with renal dysfunction in patients with obstructive jaundice. *Br J Surg* 92(11): 1388–1392. <https://doi.org/10.1002/bjs.5091>
- Pain JA, Cahill CJ, Bailey ME (1985) Perioperative complications in obstructive jaundice: therapeutic considerations. *Br J Surg* 72(12): 942–945
- Pereira, R. M., dos Santos, R. A., Oliveira, E. A., Leite, V. H., Dias, F. L., Rezende, A. S., ... Simoes e Silva, A. C. (2008). Development of hepatorenal syndrome in bile duct ligated rats. *World J Gastroenterol* 14(28), 4505–4511.
- Pinheiro SV, Simoes ESAC (2012) Angiotensin converting enzyme 2, angiotensin-(1-7), and receptor MAS axis in the kidney. *Int J Hypertens* 2012:414128–414128. <https://doi.org/10.1155/2012/414128>
- Poo JL, Estanes A, Pedraza-Chaverri J, Cruz C, Perez C, Huberman A, Uribe M (1997) Chronology of portal hypertension, decreased sodium excretion, and activation of the renin-angiotensin system in experimental biliary cirrhosis. *Rev Investig Clin* 49(1):15–23
- Raizada MK, Ferreira AJ (2007) ACE2: a new target for cardiovascular disease therapeutics. *J Cardiovasc Pharmacol* 50(2):112–119. <https://doi.org/10.1097/FJC.0b013e3180986219>
- Reich HN, Oudit GY, Penninger JM, Scholey JW, Herzenberg AM (2008) Decreased glomerular and tubular expression of ACE2 in patients with type 2 diabetes and kidney disease. *Kidney Int* 74(12):1610–1616. <https://doi.org/10.1038/ki.2008.497>
- Riabsev, V. G., & Solomka Ia, A. (1994). Combined treatment of mechanical jaundice. *Khirurgiia (Mosk)* (5): 38–42
- Richard BM (1989) Interosseous transfer of tibialis posterior for common peroneal nerve palsy. *J Bone Joint Surg Bri Vol (London)* 71(5): 834–837
- Salkind NJ, Rasmussen K (2007) Encyclopedia of measurement and statistics. SAGE Publications, Thousand Oaks

- Soler MJ, Wysocki J, Batlle D (2008) Angiotensin-converting enzyme 2 and the kidney. *Exp Physiol* 93(5):549–556. <https://doi.org/10.1113/expphysiol.2007.041350>
- Tao, K. M., Tao, Y., Chen, C. Y., Yang, L. Q., Lu, Z. J., Sun, Y. M., ..., Yu, W. F. (2014). Proteinase-activated receptor 1 contributed to up-regulation of enkephalin in keratinocytes of patients with obstructive jaundice. *Anesthesiology* 121(1):127–139. <https://doi.org/10.1097/ALN.0000000000000210>
- Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ (2000) A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem* 275(43):33238–33243. <https://doi.org/10.1074/jbc.M002615200>
- Trachtman H, Weiser AC, Valderrama E, Morgado M, Palmer LS (2004) Prevention of renal fibrosis by spironolactone in mice with complete unilateral ureteral obstruction. *J Urol* 172(4 Pt 2):1590–1594
- Ubeda M, Matzilevich MM, Atucha NM, Garcia-Estan J, Quesada T, Tang SS, Ingelfinger JR (1994) Renin and angiotensinogen mRNA expression in the kidneys of rats subjected to long-term bile duct ligation. *Hepatology* 19(6):1431–1436
- Uslu A, Tasli FA, Nart A, Postaci H, Aykas A, Bati H, Coskun Y (2010) Human kidney histopathology in acute obstructive jaundice: a prospective study. *Eur J Gastroenterol Hepatol* 22(12):1458–1465. <https://doi.org/10.1097/MEG.0b013e32833f71f6>
- Wait RB, Kahng KU (1989) Renal failure complicating obstructive jaundice. *Am J Surg* 157(2):256–263
- Wakahara S, Konoshita T, Mizuno S, Motomura M, Aoyama C, Makino Y, Kato N, Koni I, Miyamori I (2007) Synergistic expression of angiotensin-converting enzyme (ACE) and ACE2 in human renal tissue and confounding effects of hypertension on the ACE to ACE2 ratio. *Endocrinology* 148(5):2453–2457. <https://doi.org/10.1210/en.2006-1287>
- Yamamuro M, Yoshimura M, Nakayama M, Abe K, Sumida H, Sugiyama S et al (2008) Aldosterone, but not angiotensin II, reduces angiotensin converting enzyme 2 gene expression levels in cultured neonatal rat cardiomyocytes. *Circ J* 72(8):1346–1350