



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: www.elsevier.com/locate/apjtm

Document heading doi:

Protective effect of penehyclidine hydrochloride on ischemia–reperfusion injury in rats

Zhen Su¹, Wei Cai^{2*}¹Department of Anesthesiology, Huai'an First People's Hospital, Nanjing Medical University, Huai'an Jiangsu 223300, P.R. China²Department of Orthopedics, Huai'an First People's Hospital, Nanjing Medical University, Huai'an Jiangsu 223300, P.R. China

ARTICLE INFO

Article history:

Received 10 October 2013

Received in revised form 15 November 2013

Accepted 15 December 2013

Available online 20 January 2014

Keywords:

Penehyclidine hydrochloride

Renal ischemia–reperfusion injury

Inflammation factor

Radical

ABSTRACT

Objective: To investigate the protective effect of penehyclidine hydrochloride on ischemia–reperfusion injury in rats. **Method:** The model of ischemia–reperfusion injury was established in rats through clamping renal pedicles for 50 min followed by reperfusion. A total of 60 Wistar rats were randomly divided into 4 groups including fake surgery group, model group, low PHC dosage group and high dosage penehyclidine hydrochloride (PHC) group. Seven days before the experiment, rats in fake surgery group and model group were given 10 mL/kg normal saline, while rats in low PHC dosage group and high dosage PHC group were given 200 and 50 mg/kg PHC, respectively. The urine volume, diet volume, Cre, PU, BUN, IL-6, IL-8, TNF- α , NO, MDA concentration, SOD and GSH-Px were determined. **Results:** Compared with rats in model group, decreased urine volume, diet volume, Cre, PU, BUN, IL-6, IL-8, TNF- α , NO, MDA concentration and increased SOD and GSH-Px activity could be seen in low PHC dosage group and high dosage PHC group ($P < 0.05$). **Conclusions:** Administration of PHC before ischemia–reperfusion injury can help protect ischemia–reperfusion injury.

1. Introduction

Ischemia–reperfusion injury (IRI) is the tissue or organ damage due to return of blood or oxygen supply to the tissue after a period of ischemia or lack of oxygen[1]. As a high perfusion organ, kidney is an important organ of IRI. Therefore, renal ischemia–reperfusion injury (RIRI) is more common in clinical practice, which is a major incentive of kidney injury after cardiovascular surgery, trauma, or kidney transplant. RIRI can cause many kidney failure, including acute tubular necrosis, accelerated renal cell death, renal failure, delay recovery of renal function and even kidney transplant rejection[2,3]. Currently, more and more patients suffered acute kidney injury, which has drawn wide attention. Researches have shown that due to tissue hypoxia, RIRI can produce large amounts of oxygen free

radicals, which is a major cause of renal tissue damage. Therefore, oxygen free radical scavengers before RIRI can reduce the degree of injury to the kidneys.

Penehyclidine hydrochloride (PHC) is a new anti-cholinergic drug, which has a protective effect on the hemorrhagic shock with endotoxemia induced organ injury. It is highly selective to subtypes M receptor, it can also selectively antagonize the M1, M3 and N1, N2 receptors, which has a strong anticholinergic effects on central and peripheral, while it had no significant or less effect on the M2 receptors[4–7]. The protective mechanism of PHC against RIRI is not very clear. This study investigated the protective mechanism of PHC on the RIRI rats, and then explores its mechanism.

2. Materials and Methods

2.1. Experimental animals

A total of 60 female SD rats were purchased from Shanghai Experimental Animal Center, aged 3 months, weighted 150–

*Corresponding author: Wei Cai, Department of Orthopedics, Huai'an First People's Hospital, Nanjing Medical University, Huai'an Jiangsu 223300, P.R. China.
Tel: +8615052661088
E-mail: 1398549178@qq.com
Foundation project: It is supported by National Natural Science Foundation of China with grant no. 30872432.

200 g. They had free food and water during the experiment, at room temperature (22±1)°C, feeding conditions were 12 h light and 12 h darkness .

2.2. Reagents

PHC were from Chengdu List Pharmaceutical Co., Ltd., specifications 1 mg/mL; Chloral hydrate were from Tianjin Rgent Chemicals Co.; Oxidative stress parameters malondialdehyde (MDA), total superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor- α (TNF- α), nitric oxide (NO) and blood urea nitrogen (BUN) kit were products of Nanjing Jiancheng Bioengineering Institute. Proteinuria (PU) quantitative test kits and serum creatinine (Cre) kit were from BeiHua KangTai Clinical Reagent Co. products.

2.3. Main instrument

2550 UV-visible spectrophotometer (Shimadzu Corporation); high-speed refrigerated centrifuge (Shanghai Anting Scientific Instrument Factory); superfine homogenizer (Fu Luke (Shanghai) Fluid Machinery Manufacturing Co., Ltd.); Model Automatic microplate detector (Bio-Rod).

2.4. RIRI model building and grouping

A total of 60 female Wistar rats were randomly divided into sham operation group, model group, PHC low-dose group and PHC high dose group ($n=15$). Saline 10 mL/kg were given by gavage 7 d before experiments for the Sham group and model group. PHC 50 and 200 mg/kg gavage were given 7 d before the experiment, 1 time/d, the surgery was performed after 1 h of the gavage on the 7th day. The production process was as follows: rats were anesthetized with 10% chloral hydrate by intraperitoneal injection. Sham operation group only had the abdominal incision, the abdominal cavity was opened, bilateral renal artery was free, and the renal pedicle was ligated in the right kidney. Left renal pedicle was clamped with artery clip in other three groups, the artery clip was removed after 1 h, and they had reperfusion for 24 h.

2.5. Indexes detection

Before and after the experiment, the amount of daily water

and food, the weight per week of the rats were recorded, and the status of rats were observed.

After 24 h of the operation, 5 mL of blood of the rats were drawn and centrifuged (1 000 rpm \times 10 min), the serum was obtained to detecte Cre, BUN, IL-6, IL-8, TNF- α , NO and PU of the urine according to kit instructions. A small amount of kidney tissues were taken and put into saline to produce tissue homogenates (10%). It was centrifuged (3 000 rpm \times 15 min), and SOD, MDA and GSH-Px of the supernatant were measured according to kit instructions.

2.6. Statistical analysis

All data were analyzed by SPSS 17.0 software. The data were expressed as mean \pm SD values and analyzed by *t* test. $P < 0.05$ was regarded as statistically significance.

3. Result

3.1. General situation

All models were successful during the experiment. The mental and diet state of the sham-operation group were normal; The rats of the model group showed loss of luster in hair, poor mental state and shrink body; The rats of PHC high dose group and PHC low dose group were in good mental and diet state, also with luster hair and plump body. The water intake and urine volume rats in the model group were significantly higher than those in the sham-operation group ($P < 0.05$); Compared with model group, the water intake and urine volume rats in the PHC high dose group and PHC low dose group were decreased, the difference had statistically significant ($P < 0.05$) (Table 1).

3.2. Blood biochemical results

Cre, BUN and PU assay results of the serum in each group were shown in Table 2. Compared with the sham group, Cre, PU and BUN levels of the model group were significantly increased ($P < 0.05$); Compared with model group, Cre, PU and BUN concentrations of the PHC high-dose group and PHC low dose group were significantly decreased ($P < 0.05$), especially in the PHC high-dose group.

Table 1

Water intake and urine volume and weight of the rats in different groups.

Groups	<i>n</i>	Water intake (mL)	Urine volume (mL)	Weight (g)
Sham-operation group	15	118.4 \pm 2.2	6.3 \pm 0.6	172.1 \pm 4.4
Model group	15	176.4 \pm 4.2*	16.3 \pm 1.4*	178.1 \pm 3.4
PHC low-dose group	15	135.8 \pm 3.2 [▲]	9.4 \pm 1.3 [▲]	175.0 \pm 3.5
PHC high dose group	15	129.5 \pm 4.1 [▲]	10.6 \pm 1.2 [▲]	177.3 \pm 2.6

*Compared with the sham operation group, $P < 0.05$;

[▲]Compared with model group, $P < 0.05$.

Table 2

Cre, PU and BUN content in each group.

Groups	n	Cre (umol/L)	PU (mg/L)	BUN (mg/L)
Sham-operation group	15	76.46±9.04	46.23±5.44	347.34±31.36
Model group	15	153.13±2.34*	149.83±6.54*	408.24±6.30*
PHC low-dose group	15	135.63±2.45 [▲]	103.02±2.62 [▲]	399.93±21.52 [▲]
PHC high dose group	15	101.42±1.12 [▲]	89.53±4.61 [▲]	359.04±12.29 [▲]

*Compared with the sham operation group, $P<0.05$;[▲]Compared with model group, $P<0.05$.

3.3. Indicators of inflammatory cytokines

Compared with the sham group, IL-6, IL-8, TNF- α and NO levels of the model group were significantly increased ($P<0.05$); Compared with model group, IL-6, IL-8, TNF- α and NO concentrations of the PHC high-dose group and PHC low dose group were significantly decreased ($P<0.05$), especially in the PHC high-dose group (Table 3).

3.4. Renal tissue homogenates

Compared with the sham group, SOD, GSH-Px activity of the model group were significantly decreased, MDA content was increased ($P<0.05$); Compared with model group, SOD and GSH-Px activity of the PHC high-dose group and PHC low dose group were significantly increased, MDA content was decreased, the difference had statistically significant ($P<0.05$), especially in the PHC high-dose group (Table 4).

4. Discussion

RIRI can cause extensive destruction of renal units, including the decrease in glomerular filtration rate, permeability enhancement, tubular epithelial cell damage,

thus leading to the weaken of renal tubular reabsorption. The mechanism is very complex, including the generation of reactive oxygen species, apoptosis and numerous inflammatory mediators and immune factors[8-10].

RIRI is often accompanied by serum Cre and BUN and the increase of PU. The experimental results show that PHC can significantly reduce serum Cre and BUN and PU content, and has a good protect effect on glomerular and tubular. Research has shown that RIRI is accompanied by a large generation of superoxide radicals and immune cell-mediated inflammatory reaction[11-14], which indicated that superoxide radicals and numerous inflammatory factors play a key role in the process of the occurrence of RIRI.

The important inflammatory factors involved in RIRI include IL-6, IL-8 and TNF- α [15-17]. TNF- α as an important pro-inflammatory cytokines can induce the occurrence of monocyte-macrophages, neutrophils and other inflammatory factors, amplified inflammatory response; thus contribute to a large number of inflammatory cells synthesize to nitric oxide synthase, further contribute to increase of the NO concentration, cause persistent vasodilation and decrease in blood pressure, which lead to further renal ischemia and worse inflammatory injury. In addition, TNF- α may also cause the generation of peroxynitrite and hydroxyl radical, thereby cause kidney damage. And TNF- α can also causes neutrophils, eosinophils and vascular endothelial cell adhesion, block

Table 3IL-6, IL-8, TNF- α and NO content in each group.

Groups	n	IL-6 (pg/mL)	IL-8 (pg/mL)	TNF- α (pg/mL)	NO (umol/mg)
Sham-operation group	15	13.13±0.34	19.83±1.54	48.24±1.30	0.89±0.04
Model group	15	46.46±5.06*	44.23±2.47*	87.34±31.36*	1.99±0.13
PHC low-dose group	15	35.63±2.35 [▲]	33.02±2.02 [▲]	69.91±1.32 [▲]	1.54±0.18
PHC high dose group	15	24.42±3.12 [▲]	29.53±1.62 [▲]	59.04±2.29 [▲]	1.08±0.31

*Compared with the sham operation group, $P<0.05$;[▲]Compared with model group, $P<0.05$.**Table 4**

SOD, GSH-Px and MDA in each group

Groups	SOD (U/mL)	GSH-Px (U/mL)	MDA (nmol/mL)
Sham-operation group	190.14±10.92	51.35±5.46	7.01±0.18
Model group	150.83±7.25*	36.13±1.94*	14.19±0.25
PHC low-dose group	160.19±10.69 [▲]	45.53±4.15 [▲]	12.06±0.26
PHC high dose group	281.26±8.53 [▲]	49.30±1.02 [▲]	10.36±0.23

*Compared with the sham operation group, $P<0.05$;[▲]Compared with model group, $P<0.05$.

microcirculation, enhance the inflammatory response. The experimental results show that IL-6, IL-8, TNF- α and NO concentrations in the PHC high dose group and PHC low dose group were significantly reduced ($P < 0.05$), especially in the high-dose PHC group, thereby it reduce the degree of renal injury. Therefore, PHC anti-rat RIRI mechanisms may inhibit the inflammatory response.

Superoxide radical have a strong oxidation ability, it can react on the double bonds methylene of the unsaturated fatty acids of the membrane, cause the changes of membrane fluidity and permeability, also lead to mitochondrial dysfunction and plasmin leakage, then aggravate cell damage and the disorder of physical energy metabolism^[18]. MDA which generate in the process of lipid peroxidation can induced the cross-linking of membrane proteins, lipids and other macromolecules, increase cell dysfunction. When renal ischemia occurs, the increase of superoxide radicals can induce apoptosis and further aggravate kidney damage. Meanwhile, the pro-inflammatory gene expression and production of biologically active molecules can promote leukocyte activation, eventually leading to IRI. In the kidney, it was manifested as reduced glomerular filtration rate, renal tubular reabsorption dysfunction^[19,20]. Therefore, radical reactions is an important reason leading to RIRI. SOD is the specificity activating enzyme of superoxide anion, and its activity level reflects the body's ability to scavenge free radicals. This study shows that PHC can increase the activity of SOD and GSH-Px and decrease MDA levels. Therefore, the mechanism of PHC for renal protection is to inhibit lipid peroxide formation and improve the SOD and GSH-Px activity of the body, thereby enhance the protect effect on the kidneys.

In summary, this study confirmed that administration of PHC in advance before the occurrence of RIRI can reduce the kidney damage of RIRI rats, which has a significant protective effect. Its mechanism may be the inhibition of inflammatory response, scavenging free radicals and reducing rat renal tissue lipid peroxidation levels.

Conflict of interest statement

We declare that we have no conflict of interest.

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