ANIMAL RESEARCH

Berberine and Pentoxifylline: A novel combination in amelioration of acute kidney injury

Nawar Raad Hussien, Hayder M. Al-kuraishy, Ali I. Al-Gareeb

Abstract

Objectives: To evaluate the nephro-protective effects of berberine and/or pentoxifylline on the reduction of diclofenac-induced acute kidney injury in rats.

Methods: The experimental study was conducted at the Department of Pharmacology, College of Medicine, Mustansiriya University, Baghdad, Iraq, from February to April, 2018, and comprised fifty male Sprague-Dawley rats aged 3-4 months and weighing 250-400 grams each. They were divided into five equal groups and were treated for 12 days. Group 1 rats were treated with distilled water plus normal saline, Group 2 with distilled water plus diclofenac, Group 3 with berberine plus diclofenac, Group 4 with pentoxifylline plus diclofenac, and Group 5 rats were treated with berberine, pentoxifylline and diclofenac. Blood urea, creatinine, Neutrophil Gelatinase Associated Lipocalin (NGAL), kidney injury molecules (KIM-1) and cystatin-C were used to measure the severity of AKI.

Results: Diclofenac led to significant AKI by significant elevation of blood urea, serum creatinine level, KIM-1, NGAL. Treatment with berberine showed no significant effect on all biomarkers level compared to diclofenac group except on serum KIM-1 level which was also seen in pentoxifylline group. Whereas, combination of berberine and pentoxifylline led to more significant effect in reduction of all renal biomarkers.

Conclusion: Combination of berberine with pentoxifylline led to more significant reno-protective than either berberine or pentoxifylline when used alone on diclofenac induced- AKI.

Keywords: AKI, Diclofenac, Berberine, Pentoxifylline. (JPMA 69: S-93 (Suppl. 3); 2019)

Introduction

Acute kidney injury (AKI) is a renal-specific situation characterised by functional and structural renal damages, causing abrupt and rapid reduction in the renal function due to ischaemia and toxic agents. Nephrotoxic agents' frequently induce renal inflammation at glomeruli, tubular interstitial tissue and cellular matrix that lead to interstitial nephritis and glomerulonephritis. Proximal renal tubular cells are susceptible to the toxic effect of different drugs due to prolonged contact with toxic metabolites.[1]

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory, anti-pyretic and analgesic actions. Diclofenac acts through inhibition of cyclo-oxygenase (COX) enzyme, lipoxygenase pathway and phospholipase A2 which elucidate the broad anti-inflammatory effect of diclofenac.[2]

Diclofenac-induced AKI leads to acute renal injury through induction of oxidative damage, mitochondrial dysfunction and reduction of intracellular calcium concentrations at renal tubules. Thus, anti-oxidants like berberine may play a role in the prevention of diclofenac induced-AKI through attenuation of oxidative stress.[3]

Berberine (BRB) is an isoquinoline alkaloid extracted from berberis plants, and has anti-oxidant, anti-inflammatory, hepatic and renal protecting activities, as well as anti-bacterial, anti-fungal, anti-viral and immune-inducing properties.[4] BRB trims down the development of free radicals and increases levels of antioxidant enzymes with significant protection of mitochondrial membrane potential in opposition to the free radicals.[5] BRB prevents hypoxia/re-oxygenation injury of proximal renal tubular cells through modulation of endoplasmic reticulum stress pathways.[6]

Pentoxifylline (PTX) is a xanthine derivative drug used in the treatment of peripheral vascular diseases. It is useful in nephropathy by declining tumour necrosis factor-alpha (TNF-α) levels, proteinuria and micro albuminuria in diabetic patients.[7] PTX has reno-protective effects through inhibition of arachidonic acid metabolism. It can improve renal tissue injury via potential anti-inflammatory, anti-fibrotic and anti-oxidant effects, and it mitigates the development of renal injury.[8] Moreover, PTX reduces proteinuria due to the augmentation of red blood cell (RBC) deformability

Department of Clinical Pharmacology, Medicine and Therapeutic, Al-Mustansiriya University, Baghdad, Iraq.

Correspondence: Hayder Mutter. Al-kuraishy Email: hayderm36@Yahoo.com

that decreases blood viscosity, glomerular pressure and proteinuria.[9]

The current study was planned to assess the combined effect of PTX and BRB in the attenuation of diclofenac-induced AKI.

Material and Methods

The experimental study was conducted at the Department of Pharmacology, College of Medicine, Mustansiriya University, Baghdad, Iraq, from February to April, 2018, and comprised Sprague Dawley male rats aged 3-4 months and weighing 250-400 grams each. The animals, arranged from the National Centre, College of Medicine, Mustansiriya University Baghdad-Iraq, were isolated with each cage carrying 5 animals under suitable room temperature with artificial 12/12hr light-dark cycle. They were given acclimatization time of a week without any intervention and with free access to normal chow pellets and water. Humane care for animals was taken according to the Guide on the Care and Use of Laboratory Animals.[10] After the acclimatization period, the rats were weighed and randomly divided into 5 equal groups. In all the groups, the study protocol for AKI induction was in accordance with literature.[11]

Group1 rats were treated for 12 days with distilled water 2.5 ml/kg orally and on days 6-12 they received an intraperitoneal injection of normal saline 2.5 ml/kg/day. Group 2 rats were treated with distilled water 2.5 ml/kg orally for 12 days, and on days 6-12 they received an intra-peritoneal injection of diclofenac 15 mg/kg/day. Group 3 rats were treated with BRB 100 mg/kg orally for 12 days, and on days 6-12 received an intra-peritoneal injection of diclofenac 15 mg/kg/day. Group 4 rats were treated with PTX 100 mg/kg orally for 12 days and on days 6-12 received an intra-peritoneal injection of diclofenac 15 mg/kg/day. Group 5 rats were treated with PTX 100 mg/kg orally and BRB100 mg/kg orally for 12 days, and on days 6-12 received an intra-peritoneal injection of diclofenac 15 mg/kg/day.

Anthropometric variables were then assessed. Estimated glomerular filtration rate (eGFR) was measured according to Schwartz formula, eGFR =k × height (cm)/serum creatinine (mg/dl), K=0.55.[12]

Length was measured by graduated tape from nose to the anus (naso-anal length in cm). Bodyweight was measured by specific digital balance in grams. Body mass index (BMI) was taken as bodyweight in grams divided by length in cm-squared.[13]

On day 13, rat decapitation was done under chloroform anaesthesia. The blood sample was centrifuged for 5 minutes at 5000rpm at room temperature. The isolated samples were kept at -20°C till assessment.

The next step was the assessment of biochemical variables.

Blood urea and serum creatinine were estimated using an auto-analyser (ILab-300-Biomerieux Diagnostic, Milano, Italy) and they were expressed as mg/dL. Serum neutrophil gelatinase associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1) and Cystatin-c (Cyst-c) were measured by enzyme-linked immunosorbent assay (ELISA) according to the instructions mentioned on the kit by the manufacturer (Myo-bio source, USA).

Data was analysed using SPSS 20, and presented as mean ± standard deviation (SD) and the variables were tested by using unpaired student t-test between the controls and the treated groups. One way analysis of variance (ANOVA) with post-hoc test was used to scrutinise the significance of differences among the groups. Level of significance was set at $p<0.05$.

Results

Of the 50 rats, each of the five groups had 10(20%). In

Table-1: Effect of diclofenac on the anthropometric variables, biochemical and inflammatory biomarkers in diclofenac-induced acute kidney injury (AKI).

Variable	Control (n=10)	AKI (n=10)	P value
BMI (g/ cm2)	0.57±0.02	0.64±0.03	0.0003*
Blood urea (mg/dL)	41.83±7.46	70.50±12.53	0.0003*
Serum creatinine (mg/dL)	0.70±0.14	1.52±0.49	0.0019*
Estimated GFR (ml/min/1.73)	16.89±4.21	7.59±1.7	0.0001*
KIM-1(pg/mL)	73.78±16.29	269.03±29.61	0.0001*
NGAL (pg/mL)	15.78±3.07	18.76±4.13	0.16

BMI: body mass index; KIM-1: kidney injury molecule-1and NGAL: neutrophil gelatinase associated lipocalin.

Table-2: Effect of berberine (BRB) on the anthropometric variables, biochemical and inflammatory biomarkers in diclofenac-induced acute kidney injury (AKI).

Variable	AKI (n=10)	Berberine (n=10)	P value
BMI (g/ cm2)	0.64±0.03	0.54±0.01	0.0003*
Blood urea (mg/dL)	70.50±12.53	71.62±10.86	0.85
Serum creatinine (mg/dL)	1.52±0.49	1.23±0.43	0.22
eGFR (ml/min/1.73)	7.59±1.7	9.55±3.78	0.20
KIM-1(pg/mL)	269.03±29.61	89.00±29.63	0.0001*
NGAL (pg/mL)	18.76±4.13	18.13±2.95	0.73

BMI: body mass index; KIM-1: kidney injury molecule-1and NGAL: neutrophil gelatinase associated lipocalin.

Table-3: Effect of pentoxifylline (PTX) on the anthropometric variables, biochemical and inflammatory biomarkers in diclofenac-induced acute kidney injury (AKI).

Variable	AKI (n=10)	Pentoxifylline (n=10)	P
BMI (g/ cm2)	0.64±0.03	0.55±0.01	0.0001*
Blood urea (mg/dL)	70.50±12.53	64.75±27.48	0.59
Serum creatinine (mg/dL)	1.52±0.49	1.012±0.52	0.06
eGFR (ml/min/1.73)	7.59±1.7	12.22±4.33	0.01**
KIM-1(pg/mL)	269.03±29.61	71.6±31.36	0.0001*
NGAL (pg/mL)	18.76±4.13	16.78±3.79	0.33

*p<0.01; **p<0.05 unpaired t-tests, BMI: body mass index; GFR: glomerular filtration rate; KIM-1: kidney injury molecule-1; NGAL: neutrophil gelatinase associated lipocalin.

Table-4: Effect of pentoxifylline (PTX) plus berberine (BRB) on the anthropometric variables, biochemical and inflammatory biomarkers in diclofenac-induced acute kidney injury (AKI).

Variable	AKI (n=10)	Pentoxifylline+ berberine (n=10)	P
BMI (g/ cm2)	0.64±0.03	0.62±0.02	0.01**
Blood urea (mg/dL)	70.50±12.53	29.50±10.60	0.0001*
Serum creatinine (mg/dL)	1.52±0.49	0.56±0.001	0.0001*
eGFR (ml/min/1.73)	7.59±1.7	22.97±5.33	0.0001*
KIM-1(pg/mL)	269.03±29.61	42.08±7.57	0.0001*
NGAL (pg/mL)	18.76±4.13	13.37±2.85	0.008*

*p<0.01; **p<0.05 unpaired t-test, BMI: body mass index; GFR: glomerular filtration rate; KIM-1: kidney injury molecule-1; NGAL: neutrophil gelatinase associated lipocalin.

the diclofenac-induced AKI group, BMI, blood urea, serum creatinine increased significantly compared with controls (p<0.05). The eGFR was significantly reduced compared to the controls (p=0.0001).While KIM-1 significantly increased (p<0.05), serum NGAL level was non-significantly increased (p>0.05) compared to the controls (Table-1).

BRB caused significant reduction of BMI (p=0.0001) and non-significant increase in blood urea compared to the AKI group (p=0.85). Serum creatinine was decreased non-significantly in BRB group (p=0.22). The eGFR improved non-significantly (p>0.05), while KIM-1 decreased significantly (p<0.05) in the BRB group. Also, there was non-significant change in NGAL level in the BRB group compared to the AKI group (Table-2).

PTX showed significant reduction in BMI and blood urea compared to the AKI group (p<0.05), while there was non-significant decrease in serum creatinine (p>0.05). PTX significantly improved GFR and reduced KIM-1 (p<0.05) while causing significant decrease in NGAL serum level (Table-3).

The PTX-BRB combination led to significant reduction in BMI, blood urea and serum creatinine (p<0.05). The eGFR was significantly increased, while KIM-1 and NGAL were significantly reduced compared to the AKI group (Table-4).

Cyst-c level was increased significantly in AKI compared to the controls (p=0.0001). Co-administration of BRB and PTX led to a significant reduction of Cyst-c level compared to the AKI group, but it did not significantly differ compared to the controls (Figure-1).

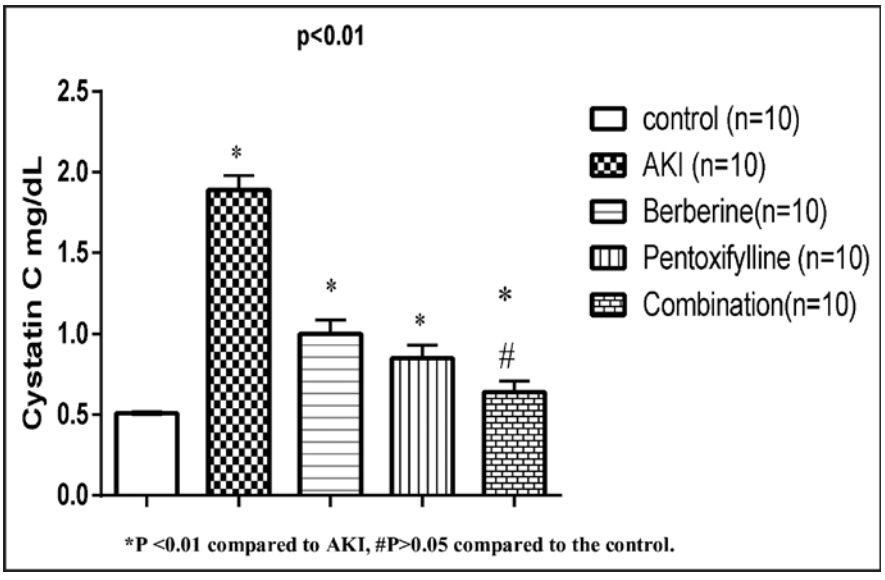

Figure: Effects of berberine (BRB) and/or pentoxifylline (PTX) on cystatin-c (Cys-c) levels in acute kidney injury (AKI).

Discussion

The present study showed significant AKI that was induced by diclofenac through the elevation of blood urea and serum creatinine compared to the controls in the experimental rats, a finding supported by literature.[14]

The induced AKI in the present study also confirmed significant decrease in eGFR which was due to the development of acute tubular necrosis and glomerular damage since short-term administration of high dose of diclofenac leads to proximal renal tubular damage and glomerular damage due to inhibition of renal prostaglandins.[15]

KIM-1serum levels were significantly correlated with different grades of

renal damage in acute nephrotoxicity and renal injury because it is highly sensitive and specific for renal tubular toxicity.[16] Co-administration of BRB with diclofenac led to significant reduction in the bodyweight and BMI compared to the AKI group due to the attenuation of diclofenac-induced AKI and the amelioration of oedematous status through the improvement of GFR, given that BRB improves renal functions with significant protection of renal tubules against diclofenac-induced renal tubules injury.[17] But blood urea and serum creatinine did not improved sufficiently which might be due to the short duration of the therapy or inadequate dose to expose the full effect of diclofenac.

On the other hand, PTX, once combined with diclofenac, led to significant reduction in the rat BMI compared to the AKI group due to the reduction of diclofenac-induced AKI and the amelioration of GFR, given that PTX improves renal functions with significant protection of renal tubules against diclofenac-induced renal tubules injury.[18]

The combined effect of BRB and PTX showed more significant ameliorative effect than BRB or PTX alone. This combination may be synergistic depending on the specific parameters that are affected. This combination led to significant reduction of BMI which might be due to the improvement of GFR and significant nephro-protective effect as reflected by the normalisation of blood urea and serum creatinine compared to the diclofenac group. These findings are consistent with a recent study that illustrated the therapeutic potential benefit of BRB use in ameliorating diabetis-induced renal dysfunction through the inhibition of renal tissue fibrosis.[19] PTX also improves renal function via reduction of oxidative stress-induced renal tubular damage.[20]

Cys-c is a sensitive biomarker for the detection of acute renal damage as it more sensitive than blood urea and serum creatinine, and, as such, it should be incorporated in the estimation of GFR.1 In the present study, Cys-c serum levels in diclofenac-induced AKI were elevated significantly compared to the controls. Co-administration of BRB and PTX led to a significant nephro-protective effect of BRB and / or PTX which was in line with previous studies.[21,22]

Conclusion

The combination of BRB and PTX led to more significant nephro-protective effect than either BRB or PTX alone in diclofenac-induced AKI.

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