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	Article History:	Submitted: 17.10.2019	Revised: 20.12.2019	Accepted: 07.01.2020
ABS sche n rei t is aggra coartic borodu co corodu corodu corodu corodu corodu corodu	ATRACT emia reperfusion injury (IR nal ischemia which is ass a popular pathological avation of tissue injury cipate in the pathophysiol uction of reactive oxygen nokine and other immun age. Previous studies ha portective, antioxidant, an erties. This study aims to DDO Me (Bardoxolone n , NF-kB signaling pathwa onstrated significant eleva hermore, activated NF-kE ased. Kidneys of pretreat	I) is a causative factor of organ failure as ociated with increasing of mortality rate. condition mainly accompanied with an . Oxidative stress and Inflammation ogy of renal IRI. This is by increasing the species, pro-inflammatory cytokines and e mediators which intensify the kidney ave demonstrated that CDDO Me has iti-inflammatory, and immunomodulating o investigate the renoprotective impacts nethyl) in rat model IRI by targeting of ay. Rats in control and vehicle groups tion in BUN, Scr, NGAL, F2-isoprostane. 8, and Nrf2 pathways, and HO-1 was ed rats with CDDO Me showed	histological and functional am reduction in BUN, Scr and isoprostane. Moreover, it w significantly more activated an when compared with cont inflammatory effects of CDD significant inhibition of NF-kE kidney damage that induced b impacts may be done by downstreamHO-1 and inhibitior Key words: IRI, NF-KB Patway, Correspondance: Najah R. Hadi Iraq DOI: 10.5530/srp.2020.1.16 © Advanced	elioration as evidenced by significan NGAL, significant reduction in F2 ras found that Nrf2 pathway was d HO-1 more expressed in renal tissue rol and vehicle groups. The anti O Me appeared clearly through the pathway. CDDO me alleviated the y bilateral renal IR, and this protective activating the Nrf2 and rising it of NF-kB pathway. Anti-Inflammatory effect

INTRODUCTION

Ischemia and reperfusion (IR) is common pathological situation often associated with exacerbation of tissue damage and a profound inflammatory response, leading to what is termed ischemia-reperfusion injury (IRI) [1]. IRI is described by restoration of blood flow to an organ after restriction and re-oxygenation [2]. IRI, occurs in many clinical settings of kidney transplantation, partial nephrectomy, hemorrhagic shock, cardiac surgery, and vascular surgery, is a major cause of AKI (acute kidney injury) [3-4]. Ischemia-reperfusion causes impairment in supplying the kidneys with oxygen and nutrient in addition to accumulation of waste products. That in turn, results in tubular endothelial cell death via different ways as apoptosis and necrosis. Subsequent, the reperfusion period, inflammatory response, oxidative stress and vascular dysfunction will further worsen the initial damage [5], restriction of blood influx to the kidney, followed by the reperfusion result in oxidative stress response that leads to overproduction of reactive oxygen species (ROS). Consequently, the ROS causes lipid peroxidation, DNA mutation, and induced apoptotic/necrotic cascades, finally leads to cell death [6-7]. The inflammatory response that occurs during IR also involves in the pathogenesis of IRI [8] by the stimulation of proximal tubules to enhance the pro-inflammatory cytokines and chemokine production. After initial ischemic injury, induction of inflammation occurs through increase in some cytokines (such as, $TNF-\alpha$, IL-1 β , IL-6, transforming growth factor β (TGF- β)), chemokine (such as, monocyte chemo tactic protein 1 (MCP-1), IL-8 and regulated on activation, chemokine (C-C motif) ligand 5 (CCL5)/ normal T cell expressed and secreted (RANTES)) that generated by tubular and endothelial cells (5). This augments the kidney injury that initiated during the ischemic period through a huge leukocyte infiltration and producing further cytotoxicity

[9]. The release kidney cytokine into the blood stream may cause external tissue injury [10]. Neutrophils gelatinaseassociated lipocalinis (NGAL) is gelatinase-associated human Neutrophils which presents as a monomer (25 kDa), as a homodimers (45 kDa) and as a heterodimeric conformation (135 kDa). Tubular epithelial cells produce both monomeric and heterodimeric forms of NGAL, while the homodimeric type occurs fundamentally in activated Neutrophils [11]. Following IRI but not in the case of pure pre-renal failure the expression level of NGAL in renal tissue elevated dramatically, this makes the NGAL one of the most promising candidates. Those data suggests the capability of NGAL to detect kidney structural injury [12]. Exposure of lipid to free radicals generates a non-enzymatic reaction cascade leading to an increased production of bioactive molecules called isoprostane. Isoprostane are prostaglandin-like compounds produced from lipid peroxidation of esterifies unsaturated fatty acids, like arachidonic acid, which are primarily produced in a nonenzymatic and free radical-dependent fashion [13]. These compounds can be liberated from phospholipids by the phospholipases A2 (PLA2) or by the platelet activating factor acetylhydrolase (PAF-AH or lipoprotein (Lp)-PLA2) [14]. Therefore, systemic isoprostane concentration is significantly raised in a variety of pathological conditions that associated with oxidative stress. Increasing of isoprostane is recognized not only as oxidative stress biomarkers but also as markers of disease progression [15-16]. The Kelch-like ECH-associated protein 1 (Keap1)nuclear factor elytroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) (Keap1/Nrf2/ARE) signaling axis acts as a "master regulator" in response to oxidative and/or electrophilic stresses or chemical insults by the induction of a large number of cytoprotective genes [17]. Therefore, the promoting of Nrf2 is deemed as a very important approach to prohibiting diseases triggered by toxins and stress [1819]. Nrf2 is a so unstable protein, usually present in conjugation with Keap1 (negative regulator), which serves as a bimolecular sensor of oxidative stress in cell. Under normal condition, Keap1 reserves Nrf2 in the cytoplasm resulting in its degradation. Especially, Keap1 serves as a protein link Nrf2 with Cul3-based E3-ubiquitin ligase complex, enhancing Nrf2 ubiquitination and resultant degradation via the 26S proteasome [20]. Dissociated Nrf2 from his suppressor translocates to the nucleus and connects its partner, small Maf protein. Ultimately the Nrf2-sMAF links ARE sequences resulting in the expression of a series of cytoprotective and antioxidant proteins [21]. Nrf2/Maf/ARE complex plays important physiological roles by its detoxification, anti-inflammatory, Autophagy, antioxidant, and proteasome actions 22] ARE binding is responsible for the regulation the expression of more than 200 genes participating in the cellular antiinflammatory and antioxidant defense like phase II detoxification enzymes (such as NADPH quinine oxidoreductase 1 (NQO1) and glutathione peroxidase), enzymes which are requisite for glutathione biosynthesis, glutathione S-transferase (GST), catalase, thioredoxin reductase, extracellular superoxide dismutase, glutathione reductase, glutamate-6-phosphatedehydrogenase, ferreting and heat shock proteins, in addition to pro- and antiinflammatory enzymes such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and Heme oxygenase-1 (HO-1) [23-24]. Heme oxygenase-1 is also recognized as heat shock protein- 32 (Hsp32). It is a ratelimiting anti-stress enzyme that destroys Heme to produce carbon monoxide (CO), biliverdin and ferrous iron. After that bilirubin reductase converts the biliverdin to bilirubin which has an antioxidant and anti-complement impact [25]. CO can act as a second messenger and regulate several cellular processing, including inflammation, proliferation, and apoptosis [26]. In addition, CO has vasodilator effect and inhibitory effect on platelet aggregation that protects from cardiovascular diseases. Consequently, HO-1 provides cytoprotective effect through its anti-apoptotic, antiinflammatory, and antioxidant properties. HO-1 also modulates the immune system by altering regulatory T cells, the function of macrophages, and dendrite cells [27]. Nuclear factor-kB (NF-kB) is a family of inducible transcription factors, which is ubiquitously expressed in all types of mammalian cell [28]. NF-kB regulates expression of hundreds of genes that are participate in growth regulation, differentiation and development of cell [29], regulation of immune responses, and can activate excessive number of genes participate in stress responses, inflammation, antioxidant, regulation of apoptosis [30] and wound healing [31]. This family consisted of 5 structurally related members, including i)NF-kB1 (also called p50), ii) NF-kB2 (also calledp52), iii) RelA (also calledp65), iv) RelB and v) c-Rel, which mediates transcription of target genes via linking to a particular DNA element, kB enhancer, as different hetero or homodimers [32]. The principal means of regulation of NF-kB activity is by retention of inactive forms in the cytoplasm of the cell via inhibitor of kB (IkB) proteins. Activation of NF-kB signaling involved two distinct pathways, the first is classical (also called canonical) pathway and the second is alternative (also called non-canonical) pathway, each leading to different biological outcomes. These pathways vary in the receptors that are able to mediate NF-kB activation, the downstream intermediate elements, and the family NF-kB members that

are then activated [33]. CDDO Me also called RTA 402 or Bardoxolone methyl is a multifunctional and mainly nontoxic anti-inflammatory, antioxidant with the ability to stimulate cytoprotective pathways, therefore it plays therapeutic roles in both oxidative stress and inflammatory process of much disease [34]. CDDOMe is more potent than parent compound (CDDO) in cancer-preventive activities and anticancer and in the activation of Keap1-Nrf2-ARE signaling pathway, which is participated in cytoprotection in the case of presence the excessive electrophilic or oxidative stress [35-36]. Oral bioavailability of CDDOMe is higher than CDDO (37). CDDOMe structure is similar to 15-deoxy- Δ 12, 14-prostaglandin J2 and related cyclopentenone prostaglandins that are endogenous Nrf2 activators that play an essential role in suppresses of NF-kB activity and resolution of inflammation [38]. Disruption of critical cysteine residues in the Keap1 protein after binding with CDDOMe result in blocking of Nrf2 ubiquitination which leads to stabilization, liberation, and cyto-nuclear translocation of Nrf2. In nucleus, Nrf2 stimulate the transcription of the phase 2 response genes, resulting in a coordinated antiinflammatory and antioxidant response [39]. In addition the CDDOMe can also bind to Cys-179 in the IKKB activation loop and suppress NF-kB activation. Linking of CDDOMe to IKKB is opposite to that in Nrf2 it will prevent the NF-kB liberation from its complex (NF-kB-IkB) in the cytoplasm, thereby suppressing NF-kB activation and downstream pro-inflammatory signaling pathways [40]. Also CDDOs have displayed potent proapoptotic and ant proliferative activity in various kinds of tumor cell lines by the suppression of MAPK (Erk1/2), PPARy and NF-kB signaling pathways and they mediate cytoprotective phase 2 response by Nrf2 signaling [41]. Gaoet al [42] reported that CDDOMe repressed T cellinduced immune responses in vivo, and revoked immune suppressive impacts in myeloid-derived suppressor cells to ameliorate immune response in cancer [43]. This indicate that CDDOMe effect on the immune system, But, little was known about immunomodulatory action of CDDOMe in vivo. The CDDOMe plays protective role against chemicalor radiation- induced fibrosis of diverse organs. Kulkarni et al [44] examined whether CDDOMe could diminish lung inflammation, fibrosis, and also lung function impairment in the model of bleomycin-induced lung damage and fibrosis. They noted that the CDDOMe had broad antiinflammatory impacts against several cytokines; include IL6 and transforming growth factor- β (TGF- β), which contribute to initiation and development of fibrosis.

MATERIALS AND METHODS

Site of Research and Time

The study was done in the department of pharmacology and therapeutics and Middle Euphrates Unit for Cancer Researches, Faculty of Medicine, and University of Kufa, Iraq from 1/12/2018 to 1/5/2019.

Animals and Ethical Considerations

Adult male Sprague Dawley rats 240-300 g were obtained from Faculty of Science / University of Zakho, Iraq. Animals were kept in animal house of Faculty of Science / University of Kufa 10 days prior to start the procedures. The animals housed under identical conditions of humidity, temperature, and 12hrs/12hrs light/dark cycles

with completely free arrival to eat and drink water, where the animals un-fasted. The study was approved by Central Committee for Bioethics at the University of Kufa and its branch in the Faculty of Medicine. All procedures were carried out according to the recommendations of the Committee.

Animal Grouping

The animals were randomly divided into 4 equal groups (6 rats in each one) and as following: Sham group: All rats subjected to same anesthetic condition, surgical protocol and same period of ischemia and reperfusion but without induction of ischemia for 30 min. and reperfusion for 2 hours. Vehicle group: The rats given only a vehicle (dimethyl sulfoxide (DMSO) 1% (v/v)) 24 hrs before surgery. CDDOMe group: Rats treated with CDDO Me 3mg/kg body weight (IP) 24 hrs before surgery. In the vehicle and CDDOMe groups the rats underwent the same procedure of ischemia-reperfusion as in control group.

Renal Ischemia-Reperfusion Injury Rat Model

Induction of ischemia done by clamping left and right renal pedicles sequentially for 30 minutes, briefly, all rats were anesthetized with an intraperitoneally injection contain mixture of ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg).The animals were placed on a heating plate to maintain body temperature constant at 37C°. Shaving the abdominal area and swabbing with antiseptic to prevent infection then cut abdominal skin after that abdominal muscle to make midline incision, clomping the two kidney pedicles for half hour with untraumatic vascular clamps. Infuse 1ml of warm saline (37C°) into the peritoneal cavity to keep rat well hydrated. After ending the ischemic time remove the clamp, suture and cover the wound with sterile gauze and wetting it with normal saline to prevent dehydration. After two hours of reperfusion the suture was opened and draw blood (6 ml) from heart then sacrificed the animals by heart puncture. The kidneys were harvest and washed with cooled phosphate buffer saline to remove blood and then cut sagittal into two halves. One of them is frozen in deep freeze after dividing into two parts for molecular study, while the other is fixed with 10% buffered formalin and embedded with paraffin for histological examination.

Preparation of Medications

The drug was prepared daily and immediately before injection by dissolving it in DMSO (1% (v/v)). CDDO Me (\geq 98% purification) has good solubility in DMSO 25.28mg/ml at 25 C°.

Assessment of Blood Parameters and Renal Function

Blood sample that drawn from each rat used to measuring BUN and Scr by using full automated biochemistry instrument (Fujifilm) that are indicators of renal function and NGAL by using sandwich ELISA Kit (Elabscinse).

Assessment of Tissue F2-Isoprostane (8-isoprostane) And HO-1

The frozen kidney tissue was divided into small pieces and washed by ice cold PBS to remove blood and clots which may affect measurements and then weight the tissue and homogenized by pestle and mortar in 1:10 (w/v) 0.1M

cooled PBS (pH 7.4) contain both 1% Triton 100X and protease inhibitor cocktail. For further degradation the homogenate was subjected to high intensity ultrasonic liquid processor. Finally the homogenate was centrifuged at 10000 rpm for 10 min. at 4 C°, the supernatant will be utilized in determination the level of both HO-1 and 8-isoprostane by using sandwich ELISA kit (Elabscinse).

Assessment of Nuclear Nrf2 and NF-kB/p65

The last part of frozen tissue will be used to extract nuclear proteins by using nuclear protein and cytoplasmic protein extraction kit (Beyotime). Briefly, after washing the tissue as mentioned above the tissue was cut into very small pieces as much as possible. Putting appropriate amount of tissue in mixture of cytoplasmic protein extraction reagents A and B that are mixed in a ratio of 20:1 (200µl of reagent an and10µl of reagent B), next, protease inhibitor cocktail was added to prepare a tissue homogenate. Tissue and tissue homogenate were mixed at a rate of 200µl of tissue homogenate per 60mg of tissue. The tissue was homogenized on the ice and centrifugated at 9000 rpm for 5 min at 4C°. The resulting supernatant was transferred to cold tube and then added 200µl of cytoplasmic reagent A which is mixed with protease inhibitor cocktail to pellet, completely suspending by highest speed vortexed for 5 seconds (can be extended the time if there is not completely suspending). Ice bath was made for 10-15 minutes then add 10µl of cytoplasmic reagent B. Again the highest speed is vortex for 5 seconds and put in ice bath for 1 minute. Centrifugation at 16,000 rpm for 5 minutes at 4C° and immediately draws the supernatant into a pre-cooled plastic tube, which is the extracted cytoplasmic protein. The pellet was resuspended in 50µl protease inhibitor cocktail-nuclear protein extraction solution and after vortex centrifugated at 16,000 rpm for 10 minutes at 4C°. The resulting supernatant was the nuclear protein portion that is used to calculate the concentration of Nrf2and NFkB/p65. Activated Nrf2 and NF-kB were assessed by measuring the level of Nrf2 and p65 in the extract of nuclear by using an Nrf2 ELISA kit (Elabscinse) and NFkB/p65 ELISA kit (PARS BIOCHEM).

Histological Analysis

Half of the left kidney immersed in 10% buffered formalin then embedded in paraffin. Tissue section of 5μ m cut by a rotary microtome and fixed on slide and stained with hematoxylin-eosin and prepared for microscopically examination. Histological evaluation of renal injury was done by two experienced pathologists who were blind to the samples taking in consideration six randomly selected fields. The sections were graded with a scale designed to assess the degree of renal damages, such as renal epithelial swelling, necrosis of tubules, losses of BB (brush border), cast formation, inflammation, vascular degeneration and desquamation. The score system which used was formed from five scores as shown in the table 2-1

Table 2-1:	Histological	scoring system	of renal	injury (49)
	0	0 /		

e	
Score	Degree (% of area damage)
0	None or Normal
1	< 25
2	25-50
3	50-75
4	>75

Statistical Analysis

All values are presented as mean \pm standard deviation (SD) of the mean. All data were analyzed with one-way analysis of variance (ANOVA)test except the histological score evaluated by using Kruskal-Wallis followed by the Mann-Whitney U test. All the statistical analyses were done by using SPSS 21 (SPSS Inc.). The result considered statistically significant when the P < 0.05.

RESULTS

Comparison of Statistical Data among Various Groups

The outcomes of statistical analysis were presented in form of diagrams for each variable among different groups.

Blood Urea Nitrogen

Comparing with sham group figure 3-1 shows that there is highly significant elevation in the level of BUN in both control and vehicle groups, while there is no significant change between them CDDO ME group appeared significant reduction in BUN when compared with control.



Figure 3-1: Blood Urea Nitrogen in the four groups

* Significant versus sham; ∞ significant versus control

Serum Creatinine

Serum analysis for creatinine concentration for the rats in control and vehicle groups shows significant increase versus the sham group (figure 3-2). Also the same analysis indicates to significant decrease in Scr in the CDDO ME group.





* Significant versus sham; ∞ significant versus control

Neutrophils Gelatinase-Associated Lipocalinis

Assessment of plasma NGAL in all groups appeared a significant elevation in control and vehicle groups (p-value 0.000 and 0.000 respectively) versus sham. While it gives

significant reduction in NGAL level when CDDO Me group compared with control group (p<0.05), shown in figure (3-3).





* significant versus sham; ∞ significant versus control.

F2-Isoprostane (8-isoprostane)

Figure 3-4 displays highly significant upswing in F2isoprostane production in control and vehicle groups (pvalue 0.000 and 0.001 respectively) comparing to sham group. In contrast, highly significant fall of F2-isoprostane in CDDO Me group versus control group.





* Significant versus sham; ∞ significant versus control

Heme Oxygenase-1

Tissue HO-1 showed significant rise in all groups comparing with sham group in addition to significant

increase in CDDO ME group versus control group. No significant change between control and vehicle groups.



Figure 3-5: Mean tissue HO-1 in the four groups.

* Significant versus sham; ∞ significant versus control

Nuclear Factor Erythroid 2-Related Factor 2

Measurement of nuclear Nrf2 level seems significant upturn in control and vehicle groups if compared with

sham group (figure 3-6). Nrf2 level in CDDO ME group has significant elevation in comparison with control group.



Figure 3-6: Mean of the Nuclear Nrf2 in the four groups

*significant versus sham; ∞ significant versus control

Nuclear Factor-kappa B/p65

Figure 3-7 explains highly significant increase in nuclear NF-kB/p65 level in both control and vehicle groups versus

sham group but in CDDO ME group there is highly significant reduction against control group.



Figure 3-7: Mean of the Nuclear NF-kB/p65 in the four groups.

* Significant versus sham; ∞ significant versus control

Histopathology

The Mean score of renal histological injury is shown in figure 3-8. In control group the score is high and in vehicle group it is slightly more than control, both groups have

significant histological change in comparison with sham group. CDDO Me group has significant reduction in tissue injury score when compared with control (figure 3-8).



Figure 3-8: Mean histopathological score in the four groups.

* Significant versus sham; ∞ significant versus control



Figure 3-9: Photomicrograph for kidney section in sham group.Normal tubules (arrows).



Figure 3-10: Photomicrograph for kidney section in control group (I/R without treatment). Tubular Necrosis (Ischemic Necrosis): appears cellular swelling, karryolysis and loss of brush border (1), Eosinophilic cast (2), Interstitial inflammation (3).



Figure 3-11: Photomicrograph for kidney section in vehicle group (I/R and treatment with DMSO): appears cellular swelling and loss of brush border (1), Pyknotic nuclei (2), interstitial inflammation (3)



Figure 3-12: Photomicrograph for kidney section in CDDO Me group (I/R and treatment with CDDO Me (3mg/kg)): shows normal tubules

DISCUSSION

Ischemia reperfusion injury involves several stresses, which include hypoxia, energy depletion, growth factor and nutrient deprivation, endoplasmic reticulum stress, genotoxicity stress, oxidant injury and other destructive insults. All of these are known to participate in death induction for epithelial cell in renal tubule, as well as inflammatory and immunological processes. Despite, the mechanistic or path physiological factors that contribute to occurrence of renal ischemia reperfusion injury is approved in large degree (though not fully understood) by several researchers in the last decades but there is no FDAapproved treatment for kidney IRI. So that, it is important to evaluate the therapeutic approaches of this problem and investigate the potential effects of some medications that have antioxidant, cytoprotective, immunomodulatory and anti-inflammatory properties which may represent promising drugs for protect the kidney from IR injury. Therefore this study was performed to assess the potential

renoprotective effect of promising drug that is CDDO Me on renal IR injury by using rat model.

Effect IRI and Drugs on Renal Function

Exposure of the kidney to 30 min ischemia and 2 hrs reperfusion result in severe nephrotoxicity change (acute and latent) at structural and functional levels. Elevation of blood urea nitrogen and serum creatinine are indicators for this change which document and reinforce by elevation of other biomarkers like cellular NGAL content. This study showed that BUN, Scr and NGAL significantly elevated in control and vehicle groups in comparison with sham group and these results corresponding with other studies. Current study indicates that CDDO Me significantly reduce BUN and Scr concentration (P<0.05) comparing with control and vehicle groups. This result supports the protective effect of studied drug to renal function where these markers are important indicators for it. To our knowledge there is no similar study to the present research but certain studies refer to that the CDDO Me reduce BUN and Scr in certain

cases like Wu et al. showed the CDDO Me protect the kidneys from induction of acute kidney injury by aristolochic acid (AA), CDDO Me administration result in improvement of renal function which appear by significant reduction in BUN and Scr levels which were increased by AAI. Also Güçlü et al., results show significant lowering of urea and total oxidant status, rise of total antioxidant status, and oxidative stress index in the CDDO ME group versus the control group. Pergola et al., in this study twenty patients complain from moderate to severe CKD and diabetes mellitus type II were assessed. Patients received 25 mg/day of CDDO Me during the first 4 weeks and 75 mg during the next 4 weeks. This study found enhancement in estimated glomerular filtration rate in 90% of patients in average of 7.2 mL/min/1.73 m2. This change paralleled a significant decrease in blood urea nitrogen and serum creatinine. Urine or plasma NGAL concentration is one of new biomarkers was recently developed for kidney injury. Despite that assessment of NGAL is more costly than BUN and Scr, but it has a valuable diagnostic performance and has a good ability to predict AKI during few hours postoperative especially in case of long warm ischemia time. So it is more advanced. This study found that CDDO Me significantly reduce plasma NGAL level (P<0.05) in comparison to control group which shows significant elevation in NGAL level in contrast to sham group. To our knowledge there is no similar study, However, Richards et al., demonstrated that IR time-dependently enhance urinary excretion of Kim-1 and NGAL in comparison to sham. CDDO Me (1 and 3 mg/kg) reduces the Kim-1 and NGAL excretion. NGAL is normally formed and secreted by tubule cells of kidney at low concentration. However, their synthesis and secretion increase dramatically by the nephron under nephrotoxicity or ischemic situations. After toxic or ischemic injury the epithelial cells of renal tubule will be damaged; NGAL level under these conditions will up regulate and liberate into the plasma and the urine; this will lead to increase plasma and urinary NGAL concentration. Apparently, the plasma NGAL would mainly arise in IRI from the injured thick ascending limb and collecting ducts of renal tubules by back-leak. Also the NGAL expression is distinctly induced in injured kidney tubular epithelial cells via NF-kB dependent pathway, which is known to be after acute epithelial cells injuries rapidly activated. This can be explained by the presence number of binding sites for many of transcription factors, like nuclear factor NF-kB in the promoter region of the NGAL gene. Furthermore, it would be expected any reduction in GFR which occurs as a result of IRI that may lead to lowering the renal clearance of the NGAL and consequently accumulate in the systemic circulation. From that it can be explained why the CDDO Me pretreated rats show significant reduction in plasma NGAL concentration when comparing with control and vehicle groups where CDDO Me and as show in this study has NF-kB inhibitory effect in addition to their cytoprotective action which result in attenuate ischemia reperfusion induce tubular injury. This study showed the important role of tested drug in attenuation of tubular injury that is associated with IRI. In addition to important of plasma NGAL level as a kidney structural and functional biomarker where accurately mirrors the extent of Tubulointerstitial damage.

Effect of IRI and Drugs on Oxidative Stress (F2-Isoprostane)

Ischemia reperfusion disturbs the redox balance, which is essential for normal renal function and leads to accumulation of ROS. This directly results in damaging of renal tubular cells by extensive peroxidation for membrane lipid, protein inactivation and DNA breakdown. Oxidative stress can quantify indirectly by measuring side-products such as protein damage markers, malondialdehyde (MDA) levels and isoprostane. In order to furthermore confirm the reno-protective effect of CDDO Me during IRI, we further tested the changing levels of the F2-isoprostane in ischemia which is consider one of promising markers of oxidative stress in the kidneys. Current study demonstrated that rats which underwent renal ischemia and reperfusion significant up regulation in tissue F2-isoprostane when compared with non-ischemic rats and this corresponding with Wang et al., and Keel et al., studies. While the rats that treated with 3 mg/kg of CDDO Me show down-regulation in F2-isoprostane level when compared with control and vehicle groups, indicating that CDDO Me abrogated the increase in oxidative stress after reperfusion. To our knowledge there is no similar study, however, Tan et al., used a novel Bardoxolone methyl derivative (dh404) at different doses to research its impacts on diabetes complications in mice were rendered diabetic by streptozotocin. The findings showed that dh404 significantly reduces oxidative stress, which represented by decrease urinary levels of 8-isoprostane and 8-hydroxy-2deoxyguanosine and plasma levels of diamicron reactive oxygen metabolites (dROMs). Diabetic male Akita mice model (insulin insufficiency and hyperglycemia), are used to examine the effects of 3 mg/kg of dh404 on endothelial function in vivo and in vitro. This study measured ROS levels by assessment two of distinct biomarkers, 8isoprostane and dROMs. Urinary 8-isoprostane levels of Akita mice appeared significantly elevated when compared with wild-type mice while significantly reduced with dh404 treatment, similarly, regarding to plasma dROMs. The results have shown that CDDO Me lower IRI-induced oxidative stress which leads to attenuate oxidative damage and this enhance the renoprotective effects of the drug.

Effect of IRI and Drugs on Nrf2/HO-1

Under stress condition, cellular antioxidants play an important role in preventing cell death by reducing oxidative stress. Activation of these antioxidants greatly alleviates the ROS elevation that associated with IRI and returns the redox balance in the ischemic kidney as become clear by reducing by products of ROS such as carbonylated protein and MDA, Nrf2 and its target genes which also known as phase-II enzymes are essential components in endogenous ant oxidative systems. HO-1 is one of these enzymes which attracted special interest because of its important therapeutic impacts for neuroprotection. HO-1 is sensitive indicator of cellular stress and induced by different stimuli. HO-1 over expression is a compensatory mechanism that saves the cells from stress as in case of ischemia, hypoxia or inflammation. Macrophages expressing HO-1 have structural and functional protective role in IRI. As in the current study many of researches refer to that exposure of tissue to ischemia and reperfusion result in activation of Nrf2 and enhancement of HO-1 expression. To explore the underlying mechanism of the protective effect of CDDO Me injured kidneys from the sham group, control group, vehicle group, CDDO Me group, rats were harvested at 2 hrs after reperfusion. Then, nucleus protein was extracted; as shown in figures 3-6, the nucleus Nrf2 concentration was significantly unregulated in the control and vehicle groups when compared with sham group. This dynamic or gradually increased nuclear translocation of Nrf2 in ischemic rats clearly indicated the response to ischemic injury at the early stage of renal IRI. Greater significant increased Nrf2 accumulation and translocation into the nucleus occurred by the CDDO Me treatment compared to the control and vehicle groups. ELISA analysis indicated a significant increase of HO-1 (in tissue homogenate) in the control and vehicle groups in comparison to sham group which enhance the early response to ischemia. CDDO Me treated rats group appeared significant elevation than in the control and vehicle groups. Wu et al. studies indicated to that CDDO Me appear Nephroprotective impact against aristolochic acid induce AKI, this protective effect that represented by improvement of renal function and ameliorate tubular damage achieved by activation of Nrf2 pathway which resulted in enhancement of HO-1 expression. Wei et al. found that treatment with CDDO Me was shown to activate Nrf2 pathway and rise antioxidant (as HO-1) gene expression in the retina in wild-type but not Nrf2 -/- mice. Also CDDOMe revoked degeneration of retinal capillary that induced by I/R in mice (wild-type, but not Nrf2 -/-). Other studies showed that CDDO Me and other related medications such as CDDO-Im increase cytoprotective genes such as peroxisome proliferator-activated receptor (PPAR), Nrf2, and HO-1 in renal tissue. The current study proved that CDDO Me treatment activate Nrf2-EAR pathway (increased Nrf2 nuclear translocation) as well as increase of HO-1 expression in ischemic kidney, suggesting that the renoprotective effect of CDDO Me in renal ischemia reperfusion might be linked with the regulation of Nrf2 and downstream target genes. It also suggested that CDDO Me has a potential effectiveness in ant oxidative reactions. This statement was verified by decreasing of F2isoprostan production from lipid peroxide in CDDO Me treated group. Moreover, HO-1 can convert Heme to beneficial bilirubin and carbon monoxide that ameliorates the deleterious effects of oxidative stress by scavenges the free radicals and repair DNA damage.

Effect of IRI and Drug on NF-kB

The inflammatory cascade regarded the main composition in the pathogenesis of renal IRI, causing tissue damage by liberation many of mediators, the kidney injury during ischemia/reperfusion period result in production of several cytokines such as TNFa, IL1 and IL6 (closely associated with renal IRI) and activates NF-kB, Neutrophils, and complement system. It is important in prevention of renal injury or treatment to target early and effectively the inflammatory response, as suppression of NF-kB ameliorate renal outcome after IR. NF-kB is a key mediator of inflammation. Efficiently NF-kB can induce the expression of chemokine, inflammatory enzymes (COX2, iNOS), adhesion molecules (VCAM-1, ICAM-1), in addition to cytokines (IL1β, IL6, TNFa,). Also stimulation of NF-kB is associated with rise of ROS generation which regulates several cellular responses. Due to these pathological roles, targeting NF-kB signaling pathway has important for the treatment numerous of inflammatory

illnesses like AKI. Several studies have demonstrated that IR is one of stressors that degraded of IkB and induce release and translocate of NF-kB p65-p50 dimer (most potent gene expression activator) so that activate this pathway. Statistical analysis in this study manifested that the nuclear expression of NF-kB/p65 protein in control and vehicle groups were significantly higher than that in sham group which correspond with Ka et al. and Zhang et al. In CDDO ME pretreated group, the NF-kB/p65 expression level was significantly lower than that in control and vehicle groups that is mean inhibition of NF-kB signaling pathway. To our knowledge there is no similar study, However, Aminzadeh et al. study refers to, in rodent models CDDO Me and close analogs (RTA dh404) demonstrate therapeutic efficacy in kidney disease. Daily administration of RTA dh404 for 12 weeks significantly attenuated NF-kB activation in addition to rise Nrf2 and its target genes expression resulted in reduced inflammation, interstitial fibrosis and glomerulosclerosis in the rats with CKD. CDDO me showed anti-inflammatory beside antioxidant response in different renal origin cells. When mesangial cells were exposed to TNFa or albumin (NF-kB activator), CDDOMe dampen NF-kB activation and expression of COX2, IL1β, and MCP-1. CDDO me demonstrated reduced NF-kB activation and expression of its target genes in glomerular endothelial cells, podocytes and proximal tubular epithelial cells. This providing evidences that CDDO Me attenuate inflammation in CKD. Based on the above results, the present study demonstrated that CDDO Me has anti-inflammatory effect which provided protective effect against renal injury during IR by NF-kB inactivation which achieved directly by reducing its nuclear translocation and indirectly by decrease ROS production.

Effect of IRI and Drug on Renal Parenchyma

In the control and vehicle groups, numerous of morphological alteration were noted by H&E staining, including necrosis of tubular cell, tubular epithelial swelling, loss of brush border and tubular impairment and obstruction. However, pretreatment with CDDO Me alleviated the intense of renal injury induced by IR. Quantitatively, the histological scores of tissue injury after 30 min renal ischemia and 2 hrs reperfusion showed significantly high when compared with sham group. But in pretreated CDDO Me group was significantly low when compared with control and vehicle groups which suggested that this kidney injury was markedly reduce by CDDO Me. Güçlü et al. pretreated rat with CDDOMe and then subjected to renal IR demonstrated marked protection, as evidenced by decreasing histological injuries in the kidney and functional improvement. Mice that received aristolochic acid appeared complaining from acute kidney injury (AKI) as manifested by decreasing in renal function and massive tubular injury CDDOMe significantly improved the renal function and attenuated the tissue injury.

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