Research Article

Ameliorative Effect of Angiotensin 1-7 (Ang1-7) on Experimentally-Induced Renal Ischemia/Reperfusion (IR) Injury and Its Remote Effects on Rat Liver

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Abstract
Aim: The present study aimed to investigate the potential protective effect of angiotensin 1-7 (Ang 1-7), the metabolic end product of angiotensin converting enzyme 2 (ACE2)/Ang (1-7)/Mas receptor arm of renin angiotensin system (RAS) on renal ischemia/reperfusion (IR) injury, as an experimental model of acute kidney injury (AKI), and its remote effects on rat liver and to explore the possible mechanisms involved. **Methods**: Twenty four adult male albino rats were divided randomly into three main groups (8 rats each); control group received no treatment; IR group, in which each rat was subjected to renal ischemia via bilateral renal artery occlusion for 45 minutes followed by reperfusion for 60 minutes, and finally IR group pretreated with a single dose of Ang (1-7), 0.3 mg/kg, i.p. At the end of the experiment, animals were sacrificed, blood samples were collected and sera were separated and used for estimation of renal and hepatic injury markers; urea, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) respectively, as well as serum ang II, ang (1-7), tumour necrosis factor-α (TNF-α), B-cell leukemia/ lymphoma-2 (Bcl-2) and nitric oxide (NO) levels. Tissue samples from both kidney and liver were also taken for histopathological examination as well as chemical estimation of malodialdehyde (MDA) and total antioxidant capacity (TAC) contents. **Results**: renal IR caused marked deterioration in both kidney and liver functions as evidenced by significantly high serum levels of renal and hepatic injury markers, Ang II and TNF-α along with significant reduction in Ang (1-7), Bcl-2 and NO levels. Tissue markers of oxidative stress were also evident in terms of significantly high MDA level along with depletion of TAC in both tissues. Histopathological examination of IR group also showed marked alteration in renal and hepatic architectures. On the contrary, Ang (1-7) treatment to IR group abolished the adverse effects of renal IR on both renal and hepatic tissues and almost reversed all the IR-induced chemical changes in both serum parameters (in terms of increased Ang (1-7), Bcl-2 and NO with significant decreases in Ang II and TNF-α serum levels and tissue parameters (as evidenced by decreased MDA level with preservation of TAC) along with marked improvement in renal and hepatic injury markers as well as histopathological alterations. **Conclusion:** renal IR has detrimental effects on kidney and liver. Ang (1-7) proved to be protective against theses adverse effects on both organs mostly *via* restoration of the normal balance of RAS system, suppression of inflammation, oxidative stress, apoptosis as well as increased NO production. Therefore, Ang-(1–7) has promise as a potential therapeutic agent in the management of renal IR injury with remote hepatic dysfunction.

Key words: renal ischemia/reperfusion (IR), kidney, liver, angiotensin (1-7), angiotensin II, inflammation, oxidative stress, apoptosis, nitic oxide (NO).

Introduction
Acute kidney injury (AKI) is a common complication that occurs in critically ill patients with considerably high morbidity and mortality rates [1, 2]. It is characterized by a sudden loss of kidney function, within hours to days, and often occurs in the setting of renal ischaemia secondary to extracellular fluid volume depletion, blood loss or sepsis[3, 4]. A hospitalized patient who develops AKI could face a mortality risk as high as 40–60% [5, 2].

A considerable body of data suggest that much of the high mortality risk associated with AKI is thought to be attributed to extra renal complications or remote organ dysfunction resulting in multi-organ failure, e.g. heart, brain and liver [6, 7, 8].

In experimental animals, the bilateral renal ischemia-reperfusion (IR) injury model, that causes both decreased renal function and ischemic organ injury, has been most commonly used to study the distant organ effects of AKI. This model is clinically relevant given that most of in-hospital AKI is thought to be ischemic in nature [7].

So far, the exact mechanism(s) of remote organ damage induced by AKI have not been fully discovered. Several potential mechanisms including dysfunctional inflammatory cascades, apoptosis, induction of remote oxidative stress, and differential molecular expression have been proposed [9, 10]. Accurate identification of these potential mechanisms and others is critical in developing targeted therapies in order to improve outcomes in AKI.

In this respect, the circulating renin-angiotensin system (RAS) is a well-recognized hormonal system, which plays an important role in the control of cardiovascular system and extracellular fluid volume. Activation of RAS has been implicated in most forms of kidney injury including AKI and inhibiting its main effector, Ang II, still remains a cornerstone of therapy [11, 12].

Until recently, a second arm of RAS involving angiotensin-converting enzyme-2 (ACE2) has been identified. This enzyme (ACE2) hydrolyses Ang II into angiotensin 1-7 [Ang (1–7)], a bioactive peptide that has vasodilatory effects and regulatory actions opposite to those of AngII [13]. It acts primarily via interaction with the G-protein-coupled receptor Mas, which is expressed in many tissues, including kidney, heart, vasculature, brain and liver [14, 15].

It has been proposed that the activation of ACE2-Ang (1–7)-Mas receptor axis may prevent or reverse organ damage in experimental models of renal diseases[16, 17]. Ferrario et al.,[18] demonstrated that increased Ang (1-7) could serve as a renoprotective factor. However, the role of Ang (1-7) in AKI and its remote hepatic injury is still unclear and needs further research.

Therefore, the present study was designed to investigate the potential protective effect of Ang 1-7 on renal IR injury, as a model of experimentally-induced AKI, and its remote effects on the liver and the possible underlying mechanisms mediating such effect in adult male albino rats. We focused on the effects of Ang (1-7) treatment at multiple levels including structural modifications, oxidative stress markers, inflammatory markers, and apoptosis.

Materials and Methods

Animals

Twenty four adult male albino rats (Sprague Dawley strain), weighing 150–200 g, about 4 months old, were included in the present study. Rats were purchased from the National Research Centre, Cairo, Egypt. Animals were housed in groups (8 rats each) in stainless steel cages to provide adequate space for free movement and wandering (40 cm × 40 cm × 25 cm) at room temperature with natural dark/light cycles, and were fed a standard diet of commercial rat chow (Nile Company, Egypt) and tap water *ad libitum* for 2 weeks for acclimatization. The protocol was ethically approved by The Laboratory Animals Maintenance and Usage Committee of the Faculty of Medicine, Minia University.

Induction of experimental renal IR

Renal IR was performed according to a previous method described by Chok et al.,[19]. In brief, anaesthesia was induced by intraperitoneal injection of xylazine (10 mg/kg) and ketamine (50 mg/kg), the anesthetized rat was placed on a heating pad to maintain the rectal temperature constant (37 ± 1°C) throughout the surgery. A midline incision was done, and the left and right renal pedicles were carefully exposed. To induce renal ischemia, both renal pedicles were bilaterally occluded using sterile non-traumatic microvascular

clamps for 45 min. Then, the clamps were removed, and the kidneys were subjected to reperfusion for 60 min. Occlusion was approved visually by colour change of the kidney to a paler shade and reperfusion by blushing.

Experimental design

The rats were randomly divided into three experimental groups (n = 8) as follows:

1. Sham-control group (C); in which all surgical procedures were done to the rats but without vascular occlusion.
2. IR-induced group (IR); in which rats were subjected to renal IR as previously mentioned.
3. IR-Ang (1-7)-treated group (IR+Ang 1-7); in which rats were pretreated with single dose of Ang (1-7) (0.3 mg/kg, i.p) (Sigma, USA) one hour before induction of IR[20].

At the end of the experiment, the rats were sacrificed by decapitation. Blood samples were collected, centrifuged, and sera were separated and stored in aliquots at −80 °C until biochemical analysis. Both kidney and liver specimens were excised, one part from each organ was weighed, stored at −80°C for further biochemical analysis. Another part was fixed in formalin for histopathological examination.

Biochemical analyses

Serum samples were used for determination of the following parameters:

- Urea, creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) by enzymatic colorimetric commercial kits (Biodiagnostic, Egypt) following the manufacturers' instructions.
- Ang II and Ang1-7 levels by ELISA (Wuhan Fine Biological Technology Co., China)
- TNF-α by ELISA method (CUSABIO, China).
- B-cell leukemia/ lymphoma-2 (Bcl-2) by enzyme-linked immunosorbent assay kit (ELISA) (Calbiotech, USA).
- Nitric oxide (NO) by enzymatic colorimetric methods using comercial kits (Biodiagnostic, Egypt).

Preparation of tissue homogenates

Specimens from both kidney and liver were weighed and homogenized separately in potassium phosphate buffer 10 mM pH (7.4). The ratio of tissue weight to homogenization buffer was 1:10. The homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for determination of both renal and hepatic contents of the following parameters:

- Malodialdehyde (MDA), as an indicator of lipid peroxidation, according to the method of Ohkawa et al.,[21].
- Total antioxidant capacity (TAC) using colorimetric assay kit according to the manufacturer's instructions (Biodiagnostic, Egypt).

Histopathological assessment

Specimens from kidney and liver were immediately fixed in 10% neutral-buffered formalin, dehydrated, cleared, and embedded in paraffin wax. Tissue sections of 5–6μm thickness were obtained and deparaffinised and were stained with haematoxylin and eosin (H&E)[22]. Sections were examined microscopically using a light microscope (Olympus, Japan). Images were digitally captured using a hardware consisting of a high-resolution colour digital camera mounted on the microscope, connected to a computer.

Statistical analysis

Data were expressed as mean ± standard error of the mean (m ± SEM). Statistical analysis was performed using Graph pad Prism V8 program (GraphPad Software, Inc. San Diego, USA) and significant difference between groups was done by one-way ANOVA followed by Tukey-Kramar post hoc test for multiple comparisons. A p value ≤ 0.05 was considered statistically significant.

Results

- **Effect of renal IR with and without Ang (1-7) administration on renal and hepatic injury markers in renal ischemic rats:**

As shown in table 1, both renal injury markers (serum urea and creatinine) as well

as hepatic injury markers (serum ALT and AST) were significantly higher in IR group than those of the control group. On the other hand, exogenous administration of Ang (1-7) reversed the condition and caused significant decrease in both renal and hepatic injury markers and brought them to the control level (Table 1).

Table 1: Effect of renal IR with and without Ang (1-7) administration on renal and hepatic injury markers in renal ischemic rats:

Parameters	Control	IR	IR+Ang (1-7)
Urea (mmol/l)	34.64±1.22	78.89±3.49[a]	36.64±1.33[b]
Creatinine (µmol/l)	0.91±0.11	10.19±1.05[a]	1.42±0.18[b]
ALT (U/ml)	19.84±1.34	83.98±3.32[a]	22.08±1.63[b]
AST (U/ml)	36.57±2.59	106.3±4.41[a]	42.24±2.36[b]

Data are expressed as mean ± SEM of eight rats/group. [a], significant difference from control group; [b], significant difference from IR group. IR, ischemia/reperfusion; Ang (1-7), angiotensin (1-7); ALT, alanine transaminase; AST, aspartate transaminase; $p \leq 0.05$.

- **Effect of renal IR with and without Ang (1-7) administration on serum levels of Ang II, Ang (1-7), TNF-α, Bcl-2 and NO in renal ischemic rats:**

Subjecting the rats to IR produced a significantly higher Ang II along with significantly lower Ang (1-7) serum levels as compared with the control group. On the other hand, treatment of IR group with exogenous Ang (1-7) significantly attenuated the IR-induced increase in serum Ang II level compared to IR non-treated rats but remained significantly higher than the control level, while, serum levels of Ang (1-7) was almost completely restored back to the control level (Table 2).

As regards the inflammatory marker, TNF-α, IR produced a significant rise in serum TNF-α level when compared with the control group, while the administration of Ang (1-7) significantly lowered the TNF-α level compared with the IR non-treated group (Table 2).

On the other hand, both Bcl-2 and NO serum levels were significantly lower in IR group as compared with the control group. However, administration of Ang (1-7) to IR rats reversed the condition and restored the levels of both parameters back to control (Table 2).

Table 2: Effect of renal IR with and without Ang (1-7) administration on serum levels of Ang II, Ang (1-7), TNF-α, Bcl-2 and NO in renal ischemic rats:

Parameters	Control	IR	IR+Ang (1-7)
Ang II	31.99±1.54	57.24±2.42[a]	40.54±1.89[ab]
Ang (1-7)	63.52±2.61	37.46±1.47[a]	58.36±2.45[b]
TNF-α (pg/ml)	14.86±1.51	51.28±3.32[a]	17.76±1.53[b]
Bcl-2 (ng/L)	68.79±2.63	48.98±1.73[a]	63.97±2.44[b]
NO (pg/ml)	32.49±2.43	14.20±1.21[a]	29.61±2.11[b]

Data are expressed as mean ± SEM of eight rats/group. [a], significant difference from control group; [b], significant difference from IR group. IR, ischemia/reperfusion; Ang (1-7), angiotensin (1-7); TNF-α, tumour necrosis factor-α; NO, nitric oxide, $p \leq 0.05$.

- **Effect of renal IR with and without Ang (1-7) administration on oxidative status in renal and hepatic tissues in renal ischemic rats:**

Renal IR induced significant rise in both renal and hepatic tissue levels of MDA along with significant decrease in TAC levels compared with the control group. Meanwhile, pretreatment with Ang (1-7) successfully attenuated the IR-induced increase in MDA and brought it back to the control level along with restoration of TAC in both renal and hepatic tissues (Table 3).

Table 3: Effect of renal IR with and without Ang (1-7) administration on oxidative status in renal and hepatic tissues in renal ischemic rats:

Parameters		Control	IR	IR+Ang (1-7)
MDA	Renal MDA	31.35±2.71	71.67±3.74[a]	39.34±2.57[b]
(pg/mg tissue)	Hepatic MDA	30.71±2.61	78.46±3.42[a]	35.15±2.24[b]
TAC	Renal TAC	67.01±2.69	47.92±3.06[a]	68.85±3.65[b]
(µM/mg tissue)	Hepatic TAC	43.61±3.14	18.31±1.50[a]	44.14±2.92[b]

Data are expressed as mean ± SEM of eight rats/group. [a], significant difference from control group; [b], significant difference from IR group. IR, ischemia/reperfusion; Ang (1-7), angiotensin (1-7); MDA, malondialdehyde; TAC, total antioxidant capacity, $p \leq 0.05$.

Histological findings in both renal and hepatic tissues induced by renal IR with and without Ang (1-7) administration:

As regards the kidney, H&E stained sections of the control group showed normal histological appearance of renal tissue, with renal corpuscles formed of tuft of glomeruli, Bowman's capsule, proximal and distal convoluted tubules. The lumina of the proximal convoluted tubules (PCTs) were narrow and lined with pyramidal cells with apical brush border and rounded nuclei. The distal convoluted tubules (DCTs) showed wider lumina and were lined with cubical cells with rounded nuclei (Fig.1; a).

Examination of renal tissue of IR group showed degeneration of renal corpuscles with widening of Bowman's space. Some renal tubular cells showed cytoplasmic vacuolations, others showed deeply eosinophilic cytoplasm and pycnotic nuclei. Some tubules were atrophied. Peritubular capillary dilatation and congestion were observed with inflammatory cell infiltration in the interstitial tissue and around blood vessels (Fig. 1; b, c, d).

On the other hand, examination of renal sections of IR+(Ang1-7) group revealed marked preservation of renal parenchyma with normal structure of renal corpuscle, decreased glomerular capillary congestion, as well as reduction of cytoplasmic vacuolations of tubular lining cells (Fig 1; e, f)

Regarding the liver tissue, examination of hepatic sections of control group showed normal hepatic architecture with hepatocytes radiating from the central vein (Fig. 2; a). In IR group, some hepatocytes appeared vacuolated, others showed apoptotic features in the form of dense nuclei and deeply acidophilic cytoplasm. There was marked vascular congestion as well as scattered inflammatory cells between degenerated hepatocytes (Fig 2; b, c, d, e). On the other hand, IR+(Ang1-7) treated group showed significantly attenuated liver damage, diminished necrosis, congestion and inflammatory cellular infiltration (Fig. 2; f).

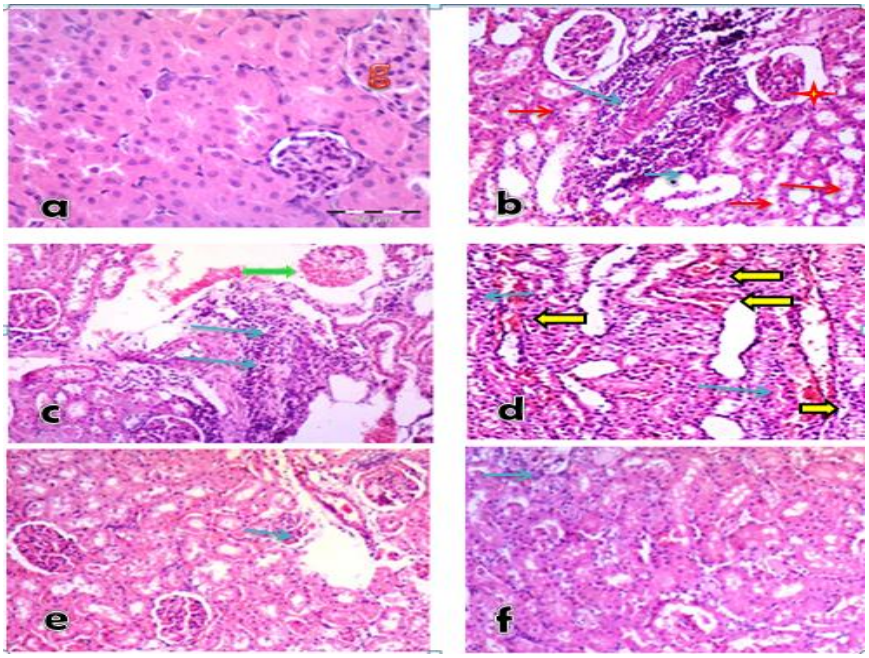

Figure 1: Photomicrograph of a section of rat kidney of: 1) control group (a), showing Malpighian renal corpuscle containing glomerulus (g) surrounded by Bowman's space, PCT is lined with high cuboidal cells having rounded basal nuclei. DCT is lined with cubical cells having rounded central nuclei. 2) IR group (b, c, d) showing: shrunken glomeruli with widening of Bowman's space (red asterisk) or even distorted (green asterisk), inflammatory cellular infiltration (blue arrows) around blood vessel in (b), around glomeruli in (c) and also between the tubules in (d). Notice also peritubular capillary dilatation and congestion in (d) (yellow arrows). 3) IR + Ang (1-7) group (e, f), the kidney tissue revealed minimal changes. (H & E x400)

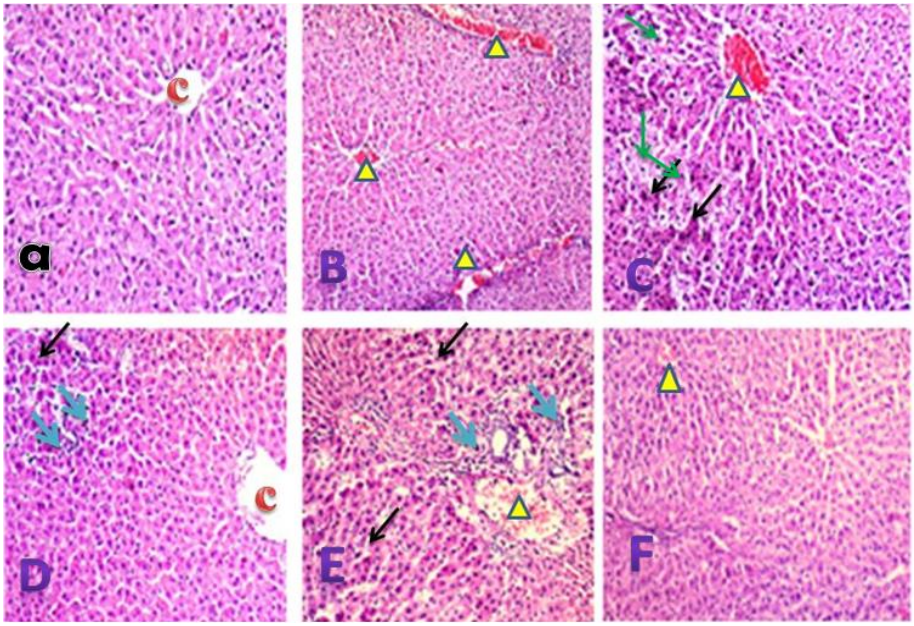

Figure 2: Photomicrograph of a section of rat liver of: 1) Control group (a), showing central vein (C), and hepatocytes radiating from it; 2) IR Group (B, C, D, E) showing, vascular congestion (yellow arrow head), inflammatory cellular infiltration between hepatocyte, and around blood vessels in portal tracts (blue arrows). Many hepatocytes show vacuolations (green arrows), deeply eosinophilic cells with pycnotic nuclei and detached (black arrows); 3) IR + Ang (1-7) group (F), show nearly normal hepatic structure with only minimal congestion and cellular infiltration (H&E X400).

Discussion

Ischemia reperfusion (IR) represents one of the common causes for AKI and it contributes considerably to a significant morbidity and mortality rates [23]. Clinically, most of the high incidence of mortality associated with AKI seems to be attributed to a systemic inflammatory response progressing into multi-organ failure including, heart, brain and liver [24, 25].

Although activation of the classic ACE1/Ang II/AT1 receptor pathway of RAS has been widely accepted as a causative factor in renal IR injury [11, 12], the role of the non-classic and potentially counter regulatory ACE2/Ang (1-7)/Mas receptor axis in renal IR and its remote organ effects is still unclear and has not been fully explored. Therefore, the present study was an attempt to evaluate the possible protective effect of exogenous Ang-(1-7); the metabolic end product of ACE2/Mas receptor arm of RAS on acute renal IR injury (as a model of AKI) and its remote effects on the liver and the possible undelaying mechanisms mediating such effect in adult male albino rats.

In the present study, subjecting the rats to renal IR resulted in a significant rise in renal injury markers; serum urea and creatinine, as well as hepatic injury markers, serum ALT and AST levels, compared to the control group. These detrimental effects of IR observed in both renal and hepatic tissues were associated with significant rises in serum levels of Ang II, and the inflammatory marker; TNF-α along with significant decreases in Ang (1-7), NO and the antiapoptotic marker; Bcl-2 serum levels. Markers of oxidative stress were also evident in both organs including elevated tissue MDA and decreased TAC contents in both kidney and liver along with marked histopathological alterations. Findings; which are in agreement with other previous reports and adding more evidence on the deleterious effects of renal IR both locally on the kidney and distantly on remote organs including the liver[26,27,28,29,10]

Renal ischemia appears to modulate the balance of RAS [30, 31]. In line with these previous reports, rats subjected to renal IR showed a significant elevation of Ang II along with a significant decrease in Ang (1-7) serum levels, indicating a shift of RAS towards increased formation of its injurious mediator Ang II on the expense its counter regulatory effector Ang (1-7), with subsequent tissue injury; which was confirmed chemically by the significant elevation in renal (urea and creatinine) and hepatic (ALT and AST) injury markers as well as histologically by the significant alterations in both renal and hepatic tissues of IR rats.

The injurious effects of elevated Ang II, observed in both renal and hepatic tissues of IR group, could be mediated through multiple mechanisms including; constriction of renal vessels, increased vascular sensitivity to sympathetic nerve stimulation [32], induction of oxidative stress [33,34], apoptosis[35] and systemic inflammation[36] with subsequent local as well as remote organ dysfunction.

A strong evidence from both laboratory and clinical studies suggests that inflammation plays a pivotal role in the progression of AKI regardless of the initiating event [36]. Biomarkers of inflammation; such as TNF-α, IL-6 are therefore of great interest in the evaluation of AKI pathogenesis and prognosis[37]. This was confirmed in the present study by the significantly high serum TNF-α levels, which could be secondary to activation of RAS and overexpression of Ang II which is known to stimulate pro-inflammatory and pro-fibrotic pathways[38]. (Ang II) has been shown to stimulate the expression of pro-inflammatory and profibrotic cytokines such as transforming growth factor (TGF) and tumour necrosis factor (TNF)[39]. Another explanation for the elevated serum TNF-α levels in IR group could be a consequence of IR-induced oxidative stress and over production of reactive oxygen species (ROS)[40], which are known for their ability to stimulate the accumulation of macrophages and monocytes in the kidney. These monocytes are the primary source of renal TNF-α[41]. The elevated TNF-α could subsequently induce a systemic

inflammatory response causing remote organ damage as observed in hepatic tissue of IR group [29, 42].

Lipid peroxidation, is a free radical-producing system, and has been tightly linked to IR-induced oxidative tissue damage[43, 40]. MDA is an important marker of oxidative stress and a good indicator of lipid peroxidation. Thus, its assessment together with the total antioxidant capacity (TAC) is an important step to ensure the involved mechanisms[44, 45]. In this study, renal IR significantly elevated the MDA level in both kidney and liver tissues. Meanwhile, the TAC contents in both tissues were significantly reduced when compared to that of the control group; Findings which are agreement with other previous studies [27, 29].

The generation of ROS during reperfusion period has been shown to poses cytotoxic effects both locally and distantly on remote organs including; DNA damage, lipid peroxidation and triggers caspase-3 activation resulting in cell apoptosis [26, 46, 47, 40]. Bcl-2 is a type of inhibitor of cell apoptosis. In the present study, the results showed a significant decrease in serum Bcl-2 level in IR group compared to that of control; findings which are in agreement with those of Abogresha et al.,[47] who reported that Bcl-2 may act as an antioxidant agent in the cell through inhibition of ROS production and preservation of mitochondrial function.

NO availability is another potential player in renal IR injury. In the present study, renal IR reduced the serum NO levels which was accompanied with deterioration in both renal and hepatic functions. Multiple studies have reported a relationship between NO and IR injury[48,49,50]. During renal IR, endothelial dysfunction occurs causing a reduction in endothelial NO synthase (eNOS) function due to a direct effect, elaboration of endogenous competitive inhibitors (e.g. asymmetric dimethylarginine, ADMA) and finally due to increased coupling of NO with superoxide, generating the more toxic peroxynitrite radical [51]. Thus, we could say that the IR-induced decrease in serum NO level, in the present study, could be attributed to either impairments in NO synthetic pathway and/or increased scavenging of NO by elevated levels of reactive oxygen species (ROS) [49].

Several potential mechanisms have been implicated in the protective effect of NO including, antioxidant mechanism by inhibiting the mitochondrial cytochrome oxidase, with subsequent decrease in free radical production [52]. Other mechanisms may involve its anti-inflammatory as well as anti-apoptotic effects probably by increasing the levels of cGMP with its potential ability to inhibit caspase-3 activity, which is involved in the induction of cell apoptosis and inflammation [48].

Collectively, based on the previous data, we can confirm the injurious effects of renal IR on both kidney as well as distantly on the liver (as a remote organ). The mechanisms of IR-induced hepatic injury, observed in the present study, seems to be multifactorial but can be summarized as follows; 1) IR-induced increase in vascular permeability, neutrophil and T-lymphocyte infiltration in the liver[46, 53]. 2) Induction of oxidative stress along with attenuated antioxidants capacity and increased expression of injury-promoting molecules, resulting in apoptosis and tissue damage of hepatocytes [27, 54]. 3) Finally, renal IR has been shown to increase small intestinal expression of interleukins resulting in small intestinal injury and subsequent cytokine flow into the liver. These events would eventually result in hepatic damage (i.e. inflammation, apoptosis, and necrosis) with increased production, and release of TNF-α and IL-6 into the systemic circulation causing further multi-organ damage [9].

In the second part of the present study, we tried to explore the potential protective effect of exogenous administration of Ang (1-7), the metabolic end product of ACE-2/Mas receptor axis of RAS on IR-induced renal and hepatic injury in rats. In the present study, IR group showed a significant reduction in Ang (1-7) serum levels along with marked deterioration in both kidney and liver functions. While, pretreatment of IR rats with exogenous Ang

(1-7) reversed the condition as it completely restored the normal renal and hepatic functions and markedly improved the histopathological alterations in both tissues. Chemically, this improvement was associated with significant reductions in Ang II and TNF-α along with significant increases in Ang (1-7), Bcl-2 and NO serum levels compared to IR-nontreated group. Oxidative status also improved significantly in both tissues as evidenced by the significant reduction in both renal and hepatic MDA levels with preservation of TAC; findings which are in agreement with other previous researches [28, 31, 13, 20].

One potential mechanism that could explain the ameliorative effect of Ang (1-7), on IR group, could be attributed to decreased serum level of the injurious Ang II, as shown in the present study. Similar results were reported by Dilauro et al.,[16] who demonstrated that treatment with Ang (1-7) significantly decreased plasma levels of AngII and suggested that Ang (1-7) could promote the degradation of Ang II and thereby provide protection against renal injury. It was proposed that the activation of the counter-regulatory RAS axis, ACE2-Ang (1-7)-Mas, could oppose the effects of the ACE-Ang II-AT1 axis and prevent or reverse organ damage. Furthermore, Kostenis et al.,[55] have reported that Ang (1-7) may act through its G-protein coupled Mas receptor and prevent the injurious effects of Ang II mediated via its AT1-receptors. Thus, it could be stated that the protective effect of Ang (1-7) on IR-induced renal and hepatic injuries could be, in part, due to increased degradation of Ang II (the injurious mediator of RAS)[56] and/or blocking its downstream effects mediated via AT1 Receptors [55].

Another possible protective mechanism for Ang (1-7) on IR injury could be mediated via its antiinflammatory effect, as evidenced in the present study, by the significant decrease in serum level of the inflammatory marker, TNF-α along with improved renal and hepatic functions in IR group pretreated with Ang (1-7). In line with these results, Fang et al.,[13] have reported that deletion of the ACE2 gene, which is responsible for the endogenous production of Ang (1-7), significantly increases cellular inflammation, pro-inflammatory cytokine expression, and apoptosis following ischemia/reperfusion (I/R). Similarly, Barroso et al.,[17] reported a renoprotective effect of AVE0991, a nonpeptide Mas receptor agonist on experimental acute renal injury. Furthermore, Khajah et al.,[57] demonstrated a potent anti-inflammatory action of Ang (1-7) against experimental colitis and this effect may be mediated through modulation of serum cytokines/chemokines levels as well as immune cell activity.

Besides suppression of inflammation, Ang (1-7) pretreatment to IR rats significantly attenuated the IR-induced oxidative injury in both renal and hepatic tissues, as evidenced by the significant reduction in MDA levels along with restoration of the antioxidant capacity with subsequent improvement in functional and histological alterations in both tissues; findings which are in agreement with other previous reports [58, 20]. Wysocki et al., [59] demonstrated that the genetic ablation of ACE2 in mice increases NADPH-mediated oxidative stress in the kidney, suggesting a potent antioxidant action of endogenous Ang (1-7), the product of ACE2, inside the body. Similarly, Mori et al.,[58] has reported that Ang 1-7 mediates renoprotection against diabetic nephropathy mostly by reducing oxidative stress, inflammation, and lipotoxicity.

Finally, in the present study, renal IR resulted in a significantly reduced serum NO level which was restored almost completely by pretreatment with exogenous Ang (1-7) along with improved renal and hepatic functions, indicating the involvement of NO as a contributing factor in the protective effect of Ang (1-7) in renal ischemic injury and its remote effects on the liver. In line with these results, Al-Maghrebi and Renno [20] reported that Ang (1-7) administration protects against testicular ischemia reperfusion injury, partially through increased NO production and this increase in NO production by Ang

(1-7) could be due to enhanced expression of constitutive nitric oxide synthase (cNOS) [60].

In conclusion, the results of the present study clearly demonstrated the effectiveness of exogenous Ang (1-7) administration in reversing all the negative effects of acute renal IR on the kidney as well as distantly on hepatic function and architecture. Proposed mechanisms may include the restoration of the normal balance of RAS system for the favour of its protective mediator Ang (1-7) and attenuating the adverse effects of its injurious mediator Ang II along with suppression of inflammation, oxidative stress and apoptosis as well as increased NO availability. Therefore, exogenous Ang-(1–7) has promise as a potential therapeutic agent in the treatment of renal IR injury with remote hepatic dysfunction.

Further research may be required using different doses of Ang (1-7) and/or combined regimens with RAS inhibitors (e.g. Ang II AT1 Blockers) to maximize their therapeutic benefit in such condition.

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التأثير الملطف للأنجيوتنسين (1-7) ضد إصابة القصور الشرياني الحاد للكلى واعادة الارتواء المحدث تجريبيا وتأثيراته البعيدة على كبد الجرذ

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الهدف: كان الهدف من البحث الحالى هو دراسة التأثير الوقائي المحتمل للأنجيوتنسين (1-7) ، المنتج النهائي الأيضي لنظام الرينين – أنجيوتنسين (RAS) من خلال أنزيمه المحول للأنجيوتنسين - 2 (ACE2) ومستقبلات ماس (Mas) على إصابة القصور الشرياني الحاد للكلى واعادة الارتواء كنموذج تجريبي لاصابة الكلى الحادة وتأثيرها البعيد على الكبد فى ذكور الجرذان البيضاء البالغة واستكشاف الآليات المحتملة المسئولة.
الطرق: استخدم فى هذا البحث أربعة وعشرون من ذكور الجرذان البيضاء تم تقسيمهم بشكل عشوائي إلى ثلاث مجموعات رئيسية (8 جرذان فى كل مجموعة) كالآتى: مجموعة ضابطة لم تتلقى أي علاج ، مجموعة القصور الشرياني الحاد للكلى واعادة الارتواء حيث تم احداث القصور الشرياني الحاد للكلى لمدة 45 دقيقة تلاه اعادة للارتواء لمدة 60 دقيقة أخرى وأخيرا مجموعة القصور الشرياني الحاد للكلى مع اعادة الارتواء والمعالجة مسبقا بجرعة واحدة من الأنجيوتنسين قدرها 0.3 مجم/كجم حقنا بريتونيا. وفى نهاية التجربة، تم التضحية بالجرذان وجمع عينات من الدم وتم فصل مصل الدم لقياس نسبة معاملات اصابة الكلى والكبد (اليوريا، والكرياتينين، وانزيمات الكبد) بالاضافة الى قياس نسب كل من الأنجيوتنسين 2 ، والأنجيوتنسين (1-7) ،ومعامل نخر الورم ألفا (TNF-α)، والبي سي ال-2 (Bcl-2) وأكسيد النيتريك فى مصل الدم. كما أخذت عينات من أنسجة الكلية والكبد لفحصها مجهريا وتحليلها كيميائيا لقياس محتواها من بيروكسيدات الدهون (MDA) والقدرة الكلية المضادة للأكسدة (TAC).
النتائج: تسبب التعرض للقصور الشرياني الحاد للكلى واعادة الارتواء الى تدهور ملحوظ فى وظائف الكلى والكبد من خلال زيادة دالة احصائيا في مستويات معاملات إصابة الكلى والكبد، الأنجيوتنسين 2، ومعامل نخر الورم ألفا (TNF-α) مصحوبا بانخفاض دال احصائيا فى مستويات الأنجيوتنسين (1-7) والبي سي ال-2 (Bcl-2) وأكسيد النيتريك فى مصل الدم. فضلا عن التغييرات المجهرية في قطاعات الكلى و الكبد والتي كانت أيضا مصحوبة بعلامات الاجهاد التأكسدى الواضحة من خلال زيادة دالة احصائيا في مستوى بيروكسيدات الدهون (MDA) مع استنزاف القدرة الكلية المضادة للتأكسد (TAC) في أنسجة الكلى والكبد. بينما تسبب العلاج بعقار الأنجيوتنسين (1-7) فى الغاء جميع التأثيرات السلبية للقصور الشرياني الحاد للكلى واعادة الارتواء بشكل شبه كامل على كل من الكلى والكبد حيث عكس تقريبا جميع التغيرات الكيميائية الناجمة عنه سواء فى قياسات مصل الدم (الواضحة من خلال زيادة مستويات الأنجيوتنسين (1-7) والبي سي ال-2 (Bcl-2) وأكسيد النيتريك مع انخفاض نسبة الأنجيوتنسين 2، ومعامل نخر الورم ألفا (TNF-α) وأيضا فى أنسجة الكلى والكبد (من خلال نقص مستوى بيروكسيدات الدهون MDA مع حفظ القدرة الكلية المضادة للأكسدة TAC، مصحوبا بتحسن ملحوظا فى التغيرات المجهرية لكلا النسيجين مقارنة بالمجموعة غير المعالجة.
الاستنتاج: نستنتج من هذة الدراسة التأثيرات السلبية للقصور الشرياني الحاد للكلى واعادة الارتواء علي وظائف الكلى والكبد. كما أثبت العلاج بعقار الأنجيوتنسين (1-7) فاعليته فى الوقاية من هذه التأثيرات السلبية على كل من أنسجة الكلى والكبد. وقد يعزوهذا التأثيرمن خلال استعادة التوازن الطبيعي لنظام الرينين أنجيوتنسين، تثبيط أليات الالتهاب، والإجهاد التأكسدي، وموت الخلايا المبرمج، بالاضافة الى زيادة إنتاج أكسيد النيتريك. ولذا فان الأنجيوتنسين (1-7) قد يعتبر واعدا كعلاج محتمل لاصابات القصور الشرياني الحاد للكلى واعادة الارتواء والمصحوبة بخلل وظيفى للكبد.