Vol 3 Issue 10 July 2014

ISSN No : 2249-894X

Monthly Multidisciplinary Research Journal

Review Of Research Journal

Welcome to Review Of Research

RNI MAHMUL/2011/38595 **ISSN No.2249-894X**

Review Of Research Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial Board readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.ror.isrj.net

Review Of Research
Vol. 3 | Issue. 10 | July. 2014
Impact Factor : 2.1002 (UIF)
ISSN:-2249-894X
Available online at www.ror.isrj.net

ORIGINAL ARTICLE

PROTECTIVE EFFECT OF GHRELIN AGAINST RENAL ISCHEMIA/REPERFUSION-INDUCED OXIDATIVE REMOTE ORGAN INJURY IN RATS

[1, 2]Elsayed Emara , [3]Ahmida H.S. Mohamed , [3]Isam Denna and [4]Esam Elshweehy

[1]Department of physiology, Faculty of Medicine, Tanta University, Egypt.
[2] Department of Laboratory Medicine, Faculty of Public Health, Benghazi University.
[3]Department of Nutrition, Faculty of Public Health, Benghazi University, Libya .
[4]Department of Histology, Faculty of Medicine, Tanta University, Egypt.

Abstract:

Background and aim: Oxygen free radicals and cytokines are considered to be important components involved in the pathophysiological tissue alterations observed during ischemia/reperfusion (I/R). Based on the anti-oxidant and anti-inflammatory effects of ghrelin, we investigated the putative protective role of ghrelin against I/R-induced oxidative remote organ injury. Materials and methods: Male albino rats were subjected to either sham operation or bilateral renal artery clamping for 45 min and reperfusion for 24 h to induce I/R damage. Ghrelin (100 µg/kg) or saline was injected subcutaneously six times before ischemia and three times after ischemia every 8 hr. At the end of the reperfusion period, the rats were decapitated and hepatic tissue were removed for biochemical analyses of: malondialdehyde (MDA), an end product of lipid peroxidation; the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT); the myeloperoxidase (MPO) activity, as an indirect index of neutrophil infiltration and the level of pro-inflammatory cytokines (TNF-α and IL-1β). The serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) levels were measured to assess liver function and tissue damage, respectively. Pathological histology was also performed. Results: The results revealed the occurrence of I/R- induced oxidative organ damage, as confirmed histologically and evidenced by an increase in the MDA level and MPO activity, and a decrease in activity of SOD and CAT. Furthermore Serum AST, ALT, LDH levels, and tissue cytokines were elevated in the renal I/R group as compared to the sham operated control group. On the other hand, ghrelin treatment succeeded to modulate these observed abnormalities resulting from I/R as indicated by the reduction of MAD and the pronounced improvement of the investigated biochemical and antioxidant parameters. Conclusion: Since ghrelin administration reversed these oxidant responses, it seems likely that ghrelin has a protective effect against oxidative organ damage induced by I/R.

KEY WORDS:

Ghrelin; Ischemia/reperfusion(I/R); Oxidative remote organ injury.

INTRODUCTION

Acute kidney injury (AKI) is a major clinical problem (Jones and Lee, 2008). However, the

morbidity and mortality from AKI is very high and remains virtually unchanged for the past 50 years in part due to a high incidence of extra-renal complications (Jones and Lee, 2008 and Bove *et al*., 2004)). In particular, hepatic dysfunction is very frequent in patients suffering from AKI. Furthermore, development of liver injury in patients with AKI frequently leads to other extra-renal complications including intestinal barrier disruption, respiratory failure and the systemic inflammatory response syndrome with the eventual development of sepsis and a multi-organ failure (Elapavaluru and Kellum, 2007; Paladino *et al*., 2009 and Grigoryev *et al*., 2008)). These extra-renal systemic complications secondary to AKI are the leading causes of mortality in the intensive care unit (Faubel, 2008). Indeed, clinical studies show that patients with isolated AKI have significantly better prognosis than patients with AKI plus extra-renal organ dysfunction (Bagshaw et al., 2005).

Ischemia reperfusion is a frequently encountered phenomenon in organisms. Prolonged ischemia followed by reperfusion results in severe oxidative injury in tissues and organs (Tan *et al*., 2005). Renal ischemia reperfusion (RIR) is a common cause of AKI (Liu *et al.*, 2008). RIR injury occurs in many clinical situations, such as transplantation, partial nephrectomy, sepsis, hydronephrosis, or elective urological operations (Kadkhodaee *et al*., 2009). Mortality during AKI is largely due to extra renal manifestations (Rabb et al., 2011 and Killy 2006).

Liver injury is one of the distant organ damage induced by RIR. Acute renal failure associated with liver disease is a commonly encountered clinical problem of varied etiology (Serteser et al., 2002). It is believed that IR injury induces inflammatory response, that elicits tissue damage in a number of organs in which reactive oxygen and nitrogen species play a key role in the pathophysiology of RIR injury (Serteser et al., 2002 and Erdogan et al., 2006). It demonstrated that RIR injury may cause liver oxidative stress and increase lipid peroxidation in liver tissue (Yildirim et al., 2003). The liver tissue of rat decreases antioxidant enzyme activities after R IR is well reported (Emre et al., 2006).

Ghrelin is a gut hormone, mainly produced in the stomach (Ariyasu *et al.*, 2001), but also identified in endocrine cells of the gastrointestinal tract (Date *et al*., 2000). Ghrelin plays a role in a number of different physiological processes. For example, it enhances growth hormone secretion (Shiiya *et al*., 2002) and increases appetite (Date *et al*., 2000), regulates cell proliferation (Yoshihara *et al*., 2002) and stimulates prolactin and adrenocorticotrophic hormone (Kojima *et al.*, 2004). In the stomach, ghrelin affects gastric acid secretion and motility, and exhibits gastroprotective effect (Brzozowski *et al.*, 2004). In mammals, ghrelin plays an important role in the immune system (Dixit *et al*., 2004). Exogenous ghrelin significantly inhibited the activation of Nuclear Factor-Kappa B (NF-kB) and plasma tumor necrosis factor alpha (TNF-α) level (Konturek *et al*., 2006).

The anti-oxidant and anti-inflammatory effects of ghrelin were previously demonstrated (El Eter *et al*., 2007 and ErŞahIn *et al.*, 2011). As the main mechanism of RIR injury is via inflammation and oxidative stress, this study was designed to evaluate the possible protective effect of ghrelin on oxidative liver injury (distant organ) induced by RIR in rats.

MATERIALS AND METHODS

Experimental Animals

All animal procedures were approved by the ethical committee of Faculty of Medicine, Tanta University. Thirty male Albino rats in the range of 230–280g body weight were used in this study. All subjects were kept in an animal room of Physiology Department of Faculty of Medicine, Tanta University, in a controlled temperature and 12:12 h light/dark cycle with free access to food and water. The animals were divided into 3 groups of 10 animals each.

Group-1: the sham group (sham operated, no I/R).
Group-2: the vehicle group (renal I/R rats treated with normal saline).
Group-3: the ghrelin group (renal I/R rats treated with ghrelin).

Surgical procedure

Rats were placed on a warming pad and anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneally). Body temperature was maintained at 37 ± 1? . A tracheotomy was performed to facilitate free breathing. The tail vein was cannulated for infusion of 0.9% NaCl solution. A midline laparatomy was performed and the renal arteries were carefully separated from around the tissues.

In the I/R groups, renal arteries were occluded by non-traumatic micro-vascular clips for 45 min,

followed by 24 hr. reperfusion. Occlusion was approved visually by color change of the kidney to a paler shade and reperfusion by blushing. Sham operated animals underwent identical surgical treatment, including isolation of both renal arteries. However, artery occlusion was not performed. At the end of the experimental procedure, the animals were decapitated and trunk blood samples were collected to determine serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) levels as indicators of liver function and damage, respectively. Liver samples were taken and stored at - 70 °C for the measurement of Pro-inflammatory cytokines (TNF-a and IL-1ß) and malondialdehyde (MDA) levels, superoxide dismutase (SOD), catalase (CAT) and myeloperoxidase (MPO) activities were measured. Additional liver samples were fixed with 10% formaldehyde for histopathological evaluation.

Administration of Ghrelin

Rat ghrelin (Sigma Chemical, St. Louis, MO) was dissolved in1ml of 0.9% physiological saline and injected subcutaneously at a dose of (100 µg/kg) six times before ischemia every 8 hr. and three times after ischemia. This dose of ghrelin was determined from a previous model of injury (Takeda *et al.*, 2006). An equal volume of the saline was injected into the vehicle rats. The sham group of animals only underwent laparotomy.

Estimation of liver function and damage

Serum AST, ALT and LDH activities were determined to assess the liver damage by using AST, ALT and LDH (Roche Diagnostic, Mannheim, Germany) commercial kits in a Roche - Hitachi Modular Auto analyzer (Roche Diagnostic).

Estimation of cytokine concentrations

TNF-a and IL-1ß levels were determined by using a commercially available rat ELISA kit (MedSystems Diagnostics GmbH, Vienna, Austria) and the results were expressed as pictograms per milligram protein.

Estimation of lipid peroxidation and antioxidant enzymes

Malondialdehyde(MDA) was determined in liver homogenates using commercial kits (LPO-585) from Bioxytec (Bioxytec, Portland, USA). Superoxide dismutase (SOD) and catalase (CAT) activities were measured according to Sun et al. (1988) and Luck et al. (1965) respectively.

Determination of myeloperoxidase activity

Tissue samples (0.2–0.3g) were homogenized in 10 volume of ice-cold potassium phosphate buffer (50 mmol/L K_2HPO_4, pH 6.0) containing hexadecyltrimethylammonium bromide (HETAB; 0.5%, w/v). The homogenate was centrifuged at 30,000 g for 10 min at 4°C, and the supernatant was discarded. The pellet was then rehomogenized with an equivalent volume of 50mmol/L K_2HPO_4 containing 0.5% (w/v) HETAB and 10 mmol/L ethylenediaminetetraacetic acid (EDTA, Sigma). MPO activity was assessed by measuring the H_2O_2-dependent oxidation of o-dianizidine 2HCl. One unit of enzyme activity was defined as the amount of the MPO present per gram of tissue weight that caused a change in absorbance of 1.0/min at 460 nm and 37 °C (Hillegas et al., 1990).

Histopathological evaluation

For light microscopic examinations, liver samples were fixed in 10% neutral buffered formalin solution. The tissues were embedded in paraffin. The paraffin blocks were cut in 5 µm thick. The sections were stained with Hematoxylin-Eosin (H&E). All tissue sections were examined microscopically for the characterization of histopathological changes by an experienced histologist in blind fashion.

Statistical analysis

All the values are expressed as mean ± SEM. Statistical significance between more than two groups were tested using one-way ANOVA followed by the Bonferroni multiple comparisons test using

computer based fitting program (Prism, Graphpad 5). Differences were considered to be statistically significant when $P < 0.05$.

RESULTS

Effect of ghrelin on liver function and damage

Renal IR-induced liver injury caused significant increases in the ALT and AST levels indicating impairment of liver functions. Similarly, LDH activity, as an index of generalized tissue damage was also found to be increased compared with the control. When ghrelin was administered before ischemia and during the subsequent reperfusion period, these elevations were significantly depressed ($P < 0.01–0.001$) (Table 1).

Table 1: Changes in serum alanin amimotransaminase (ALT), aspartate amimotransaminase(AST) and lactate dehydrogenase (LDH) levels in the Sham, Vehicle (RIR + saline) and Ghrelin (RIR+GHR) Groups.

Groups	Measured parameters		
	ALT (U/L)	AST (U/L)	LDH (U/L)
Sham group	49 ±4.3	92 ± 6.2	544 ± 95
Vehicle group (RIR + saline)	263 ± 9.9***	227 ± 16.5***	1342 ±149***
Ghrelin group (RIR+GHR)	128 ± 5.7+++,**	141 ± 6.1+++,*	818 ±78++

Each group consists of ten rats; Data are expressed as mean S.E.M.; *P < 0.05; **P <0.01, ***P < 0.001: compared with sham group; ++P < 0.01; +++P < 0.001: compared with vehicle group.

Effect of ghrelin on lipid peroxidation and antioxidant enzymes

The liver tissue MDA content in the sham operated control group was elevated by renal I/R injury ($P < 0.01$); however, ghrelin treatment significantly decreased the renal I/R-induced elevation in liver MDA level ($P < 0.01$; Table 2 and Fig. 1). In accordance with this, renal I/R caused a significant decrease in the activities of hepatic anti-oxidant enzymes (SOD) and (CAT) ($P < 0.01$; $P<0.05$, respectively) compared with sham operated control group, while in the ghrelin-treated renal I/R group, activities of hepatic anti-oxidant enzymes (SOD) and (CAT) was found to be preserved significantly ($P < 0.01$; $P<0.05$, respectively), not significantly different from that of the control group (Table 2).

Table 2: Changes in liver MDA level and SOD and CAT activities in the Sham, Vehicle (RIR +saline) and Ghrelin (RIR+GHR) Groups.

Groups	Measured parameters		
	MDA (nmol/mg-protein)	SOD (U/mg-protein)	CAT (U/mg-protein)
Sham group	2.71 ± 0.67	84.6 ±7. 8	19.93 ± 2.08
Vehicle group (RIR + saline)	6.12 ± 1.24**	52.7 ± 17.8**	12.24 ± 1.43*
Ghrelin group (RIR+GHR)	3.94 ± 1.13++	71.8 ± 12.7++	17.31 ± 1.35+

Each group consists of ten rats; Data are expressed as mean S.E.M.; *P < 0.05; **P <0.01 compared with sham group; +P ? 0.05; ++P < 0.01 compared with the vehicle group.

Effect of ghrelin on cytokine concentrations

Pro-inflammatory cytokines (TNF-α and IL-1ß) levels were significantly higher in the liver tissue of the vehicle saline-treated I/R group ($P < 0.001$) than that of the sham- operated control group (Table 3). On the other hand, ghrelin treatment in the I/R group decreased the elevations in levels of pro-inflammatory cytokines (TNF-a and IL-1ß) significantly ($P < 0.01–0.001$)

Table 3: Changes in hepatic TNF-a, and IL-1ß Levels in the Sham, Vehicle (RIR + saline) and Ghrelin (RIR+GHR) Groups.

Groups	Measured parameters	
	TNF-α (pg/mg)	IL-1β (pg/mg)
Sham group	1.4 ± 0.2	10.2 ± 1.2
Vehicle group (RIR +saline)	8.2 ± 0.8***	19.4 ±1.1***
Ghrelin group (RIR+GHR)	4.8 ± 0.5**, ++	10.7 ± 0.7+++

Each group consists of ten rats; Data are expressed as mean ± S.E.M.; **P < 0.01; ***P < 0.001 compared with the Sham group; ++P ? 0.01; +++P < 0.001 compared with the Vehicle group.

Effect of ghrelin on myeloperoxidase activity

Myeloperoxidase activity, which is accepted as an indicator of neutrophil infiltration, was significantly higher in the liver tissue of the vehicle saline-treated I/R group (P < 0.001) than that of the sham - operated control group (Table 4). On the other hand, ghrelin treatment in I/R group significantly decreased liver tissue MPO level (P < 0.001).

Table 4: Changes in myeloperoxidase activity in the Sham, Vehicle (RIR +saline) and Ghrelin (RIR+GHR) Groups.

Groups	Myeloperoxidase (U/g)
Sham group	10.6 ± 0.6
Vehicle group (RIR +saline)	21.1 ±1.1***
Ghrelin group (RIR+GHR)	14.6 ± 1.1*,+++

Each group consists of ten rats; Data are expressed as mean ± S.E.M.; *P < 0.05; ***p < 0.001 compared to the sham group; +++p < 0.001 compared with the Vehicle group.

Histopathological study

In the vehicle group (RIR + saline), hepatocyte necrosis areas randomly disseminated in the liver parenchyma with disintegrated cell cordons, leucocyte infiltration, expansion of sinusoids and congestion were detected. Hepatocyte damage was not observed in the liver parenchyma of the ghrelin group (RIR+GHR) except for isolated necrotic hepatocytes. Sinusoid expansion was much decreased compared to the vehicle group (RIR + saline). These observations are illustrated in Fig. (1).

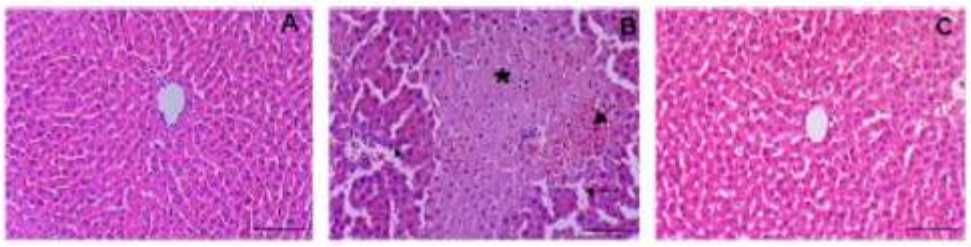

Fig. 1: The effect of ghrelin treatment in the liver of rats (hematoxylin and eosin stain). Representative photomicrographs of hepatocyte necrosis (*), congestion (?), expansion of sinusoid (⟵): (A) sham group, (B) vehicle group (RIR +saline), (C) ghrelin group (RIR+GHR) [scale bar = 100 µm].

DISCUSSION

The present study demonstrated that ghrelin administration in RIR-induced hepatic injury improved the liver functions and decreased the elevations in serum LDH activity and pro-inflammatory

cytokine levels. Furthermore, increased myeloperoxidase activity and hepatic lipid peroxidation level and decreased activity of antioxidant enzymes that were observed as the consequences of oxidative injury were also reversed by ghrelin treatment. Histopathologic findings also supported the anti-inflammatory effects of ghrelin in RIR-induced hepatic damage. Ischemia/reperfusion injury is a complex process involving numerous intracellular signaling pathways, mediators, cells and pathophysiological disturbances; its prevention during surgery is of utmost importance (Sakon *et al*., 2002). It has been suggested that I/R triggers a series of reactions mainly in the organ which is clamped and reperfused, and these reactions elicit a systemic inflammatory response by the release of cytokines and inflammatory mediators (tumor necrosis factor-α, interleukin-6, nitric oxide, etc.) which cause the generation of free radicals (Serteser *et al*., 2002; Rabb *et al*., 2000 and Yassin *et al.*, 2002). Inflammatory mediators released as a consequence of reperfusion activate endothelial cells and circulating neutrophils in remote organs that are not exposed to the initial ischemic insult. This distant response to I/R can result in leukocyte-dependent microvascular injury that is characteristic of multiple organ dysfunction syndrome (Carden & Granger, 2000 and Khastar *et al*., 2011). Recently, it has been demonstrated that acute kidney ischemia causes small intestinal generation of IL-17A and subsequent intestinal injury (villous endothelial apoptosis, epithelial necrosis, increased pro-inflammatory cell translocation and cytokine flux to the liver). These events cause hepatic injury (inflammation, apoptosis and necrosis) with increased generation and release of TNF-a and IL-6 systemically causing further multi-organ injury and systemic inflammation (Park et al., 2011). As a result, in this study, although the liver tissue specimen was not exposed to ischemia directly, we aimed to investigate whether I/R caused remote organ injury as a result of a systemic effect.

Several studies have demonstrated that ischemia and reperfusion of the tissue to be associated with lipid peroxidation, which is an autocatalytic mechanism leading to oxidative destruction of the cellular membranes,and their destruction can lead to the production of toxic, reactive metabolites and cell death (Eschwege et al., 1999). Lipid peroxidation, as a free radical-generating system, has been suggested to be closely related to I/R-induced tissue damage, and MDA is a good indicator of the rate of lipid peroxidation. In the present study, the levels of MDA significantly increased due to I/R. This observation correlates with previous studies, in which the elevated levels of lipid peroxidation products increased from 40% to 100% above the basal values (Serteser **et al.**, 2002 and Sumimoto *et al.*, 1990). Our results show that ghrelin caused a significant inhibition in MDA production, thus indicating a reduction in lipid peroxidation and cellular injury. This result agrees with previous findings showing that ghrelin induced reduction of the increased level of MDA in preadipocyte cell culture (Zwirska *et al*., 2007) testis and ovary of rats (Kheradmand *et al.*, 2009 and Kheradmand *et al.*, 2010). Similarly, Obay *et al.* (2008) demonstrated that pretreatment of rats with different doses of ghrelin prevented pentylenetetrazole-induced elevation in lipid peroxidation. Likewise, Işeri *et al*. (2005) showed that ghrelin administration significantly decreased MDA level in the alendronate-induced gastric tissue injury in rats.

Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, and hydrogen peroxide, have a causal relationship with oxidative stress. Antioxidant enzymes such as SOD and CAT represent protective response against oxidative tissue-damage. SOD converted superoxide anion into H_2O_2. Catalase metabolizes H_2O_2 to water. Maintaining the balance between ROS and antioxidant enzymes is therefore crucial, and could serve as a major mechanism in preventing damage by oxidative stress (Halliwell and Gutteridge, 1990). In the present study, we observed a decrease in the activities of SOD and CAT in liver induced by RIR. This decrease may be attributed to oxidative inactivation of enzyme protein by lipid peroxyl radicals and excess ROS generation. Treatment with ghrelin showed an improved effect on the activities of antioxidant enzymes examined as compared to vehicle group. Our results confirm previous data concerning the antioxidative properties of ghrelin in other tissues (Kheradmand *et al*., 2010; liu *et al.*, 2010 and Cetin *et al*., 2011).

The pro-inflammatory cytokines, TNF-α and IL-1ß, are known to play pivotal roles in the pathogenetic mechanisms of remote organ injury (Aikawa, 1996). Following experimental AKI, hepatic TNF-a, IL-6 and IL-17A are increased (Park et al., 2011 and Golab et al., 2009). Knockout mice for TNF-a, IL-17A, and IL-6 displayed reduced hepatic injury after renal ischemia, as did wild-type mice treated with antibodies to the cytokines (Park *et al*., 2011). Konturek *et al.* 2006 reported that ghrelin significantly attenuated ischemia/reperfusion-induced gastric lesions and accelerated the healing of these lesions, by significantly raising the gastric blood flow and by inhibiting the activation of NF-kappa B and plasma TNF-a levels. Similarly, ghrelin was shown to protect the kidneys from ischemia/reperfusion injury (Takeda *et al.*, 2006) and to ameliorate oxidative gastric damage while decreasing serum TNF-a levels (Işeri *et al*., 2005). In accordance with these observations, the findings of the current study show that ghrelin treatment ameliorated RIR-induced oxidative hepatic injury by a mechanism that involves an inhibitory effect on the release of inflammatory cytokines. Interestingly, a recent study revealed that ghrelin significantly reduced TNF-α and IL-6 levels and down-regulated pro-inflammatory cytokines in rats with RIR injury through the

activation of the vagus nerve (Rajanc *et al*., 2012), suggesting the presence of ghrelin receptors on the vagal fibers to mediate its anti-inflammatory action. Liver dysfunction and cell injury induced by neutrophils have been demonstrated in a number of experimental models, including ischemic reperfusion injury (Jaeschke and Hasegawa, 2006). Several methods have been used to define the role of neutrophils in the tissue injury. One of them is to measure the activity of neutrophil-specific enzyme, MPO, which is released as a response to various stimulatory substances (Kettle and Winterbourn, 1997). In the present study, increased hepatic MPO activity due to RIR indicates that liver injury involves the contribution of neutrophil infiltration. Since ghrelin depressed the tissue MPO activity in concomitant with its anti-inflammatory effect, it appears that the mode of action of ghrelin treatment in hepatic injury involves its inhibitory effect on tissue neutrophil infiltration that limits neutrophil-derived oxidative tissue damage.

In the present study following I/R injury, plasma ALT, AST, and LDH levels were increased indicating impairment of liver functions and generalized tissue damage, respectively, while these increases were significantly reduced by ghrelin treatment. Our findings were also confirmed by histological observation. Hepatocyte necrosis areas randomly disseminated in the liver parenchyma with disintegrated cell cordons, leucocyte infiltration, expansion of sinusoids and congestion were displayed after RIR. Treatment with ghrelin markedly reduced these histopathological changes.

In conclusion, since the administration of ghrelin inhibited the release of the pro-inflammatory cytokines and the accumulation of neutrophils in the damaged hepatic tissue, these agents appear to play a cytoprotective role in the liver insulted by I/R. It seems likely that ghrelin, with its efficiency as antioxidant and antinflammotory, thus merits consideration as a potential therapeutic agent against I/R-induced oxidative remote organ damage.

REFERENCES

1.Aikawa, N. 1996. Cytokine storm in the pathogenesis of multiple organ dysfunction syndrome associated with surgical insults. Nippon Geka Gakkai Zasshi, 97: 771.

2.Ariyasu, H.; Takaya, K.; Tagami, T., Ogawa, Y.; Hosoda K.; Akamizu T. et al. 2001. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. Journal of Clinical Endocrinology & Metabolism, 86: 4753–8.

3.Bagshaw, S.M.; Laupland, K.B.; Doig, C.J.; Mortis, G.; Fick, G.H.; Mucenski, M.; Godinez-Luna, T., Svenson, L.W. and Rosenal, T. 2005. Prognosis for long-term survival and renal recovery in critically ill patients with severe acute renal failure: a population-based study. Crit. Care; 9: R700–R709. [PubMed:16280066].

4.Bove, T., Calabro, M.G., Landoni, G., Aletti, G., Marino, G., Crescenzi, G., Rosica, C. and Zangrillo, A. 2004. The incidence and risk of acute renal failure after cardiac surgery. J Cardiothorac Vasc. Anesth., 18: 442–445. [PubMed: 15365924]

5.Brzozowski, T, Konturek P, Konturek S, Kwiecie S, Drozdowicz D, Bielanski W. et al. 2004. Exogenous and endogenous ghrelin in gastroprotection against stressinduced gastric damage. Regulatory Peptides; 120: 39–51.

6.Carden, D.L. and Granger D.N. 2000. Pathophysiology of ischemia-reperfusion injury. J Pathol; 190: 255–66.

7.Cetin, N, Boyroz U and Cetin E. Ghrelin 2011. Alleviates Tilmicosin-Induced Myocardial Oxidative Stress in rats. Journal of Animal and Veterinary advances, 10(15): 2038-2042.

8.Date, Y, KojimaM, Hosoda H, Sawaguchi A, Mondal M, Suganuma T. et al. 2000. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology, 141: 4255–61.

9.Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, Lillard Jr JW. and Taub DD. 2004. Ghrelin inhibits leptin-and activation-induced proinflammatory cytokine expression by human monocytes and T cells. J. Clin. Invest., 114: 57–66.

10.El Eter, E, Al Tuwaijiri A, Hagar H. and Arafa M. 2007. In vivo and in vitro antioxidant activity of ghrelin: attenuation of gastric ischemic injury in the rat. Journal of Gastroenterology and Hepatology, 22: 1791–9.

11.Elapavaluru, S. and Kellum JA. 2007. Why do patients die of acute kidney injury? Acta. Clin. Belg. Suppl., 326–331. [PubMed: 18283993].

12.Emre, MH, Erdogan H. and Fadillioglu E. 2006. Effect of BQ-123 and nitric oxide inhibition on liver in

rats after renal ischemia-reperfusion injury. Gen. Physiol. Biophys., 25: 195-206.
13.Erdogan, H, Fadillioglu E, Yagmurca M. et al. 2006. Protein oxidation and lipid peroxidation after renal ischemia reperfusion injury: protective effects of erdosteine and Nacetylcysteine. Urol. Res., 34: 41-46.
14.ErŞahIn, M, Toklu HZ, Can ErzIk C. et al. 2011. Ghrelin Alleviates Spinal Cord Injury in Rats Via Its Anti-inflammatory Effects. Turkish Neurosurgery, 21(4): 599-605
15.Eschwege, P, Paradis V, Conti M, Holstege A, Richet F. and Deteve J. et al. 1999. In situ detection of lipid peroxidation by-products as markers of renal ischemia injuries in rat kidneys. J Urol; 162: 553–7.
16.Faubel, S. 2008. Pulmonary complications after acute kidney injury. Adv Chronic Kidney Dis., 15: 284–296. [PubMed: 18565479]
17.Golab, F, Kadkhodaee M, Zahmatkesh M. et al. 2009. Ischemic and non-ischemic acute kidney injury cause hepatic damage. Kidney Int., 75: 783–792.
18.Grigoryev, DN, Liu M, Hassoun HT, Cheadle C, Barnes KC and Rabb H. 2008. The local and systemic inflammatory transcriptome after acute kidney injury. J. Am. Soc. Nephrol.; 19: 547–558. [PubMed: 18235097]
19.Halliwell, B. and Gutteridge J.M.C. 1990. Role of free radicals and catalytic metal irons inhuman disease: an overview. Methods Enzymol., 186: 59–85.
20.Hillegas, LM, Griswold DE, Brickson B. and Albrightson-Winslow C. 1990. Assesment of myeloperoxidase activity in whole rat kidney. J Pharmacol Methods; 24: 285–95.
21.Iseri, SO, Sener G, Yüksel M, Contuk G, Cetinel S, Gedik N. and Yegen BC. 2005. Ghrelin against alendronate-induced gastric damage in rats. J. Endocrinol., 187: 399–406.
22.Jaeschke, H. and Hasegawa T. 2006. Role of neutrophils in acute inflammatory liver injury. Liver Int., 26: 912–9.
23.Jones, D.R. and Lee HT. 2008. Perioperative renal protection. Best Pract. Res. Clin. Anaesthesiol., 22: 193–208. [PubMed: 18494397].
24.Kadkhodaee, M, Golab F, Zahmatkesh M. and Ghaznavi R. 2009. Effects of different periods of renal ischemia on liver as a remote organ. World J. Gastroenterol., 15(9): 1113–1118.
25.Kelly, K.J. 2006. Acute renal failure: much more than a kidney disease. Semin Nephrol., 26: 105–113,
26.Kettle, A.J. and Winterbourn C.C. 1997. Myeloperoxidase: a key regulator of neutrophil oxidant production. Redox. Rep., 3: 3–15.
27.Khastar, H, Kadkhodaee M, Sadeghipour HR et al. 2011. Liver Oxidative Stress after Renal Ischemia-Reperfusion Injury is Leukocyte Dependent in Inbred Mice. Iranian Journal of Basic Medical Sciences, 14(6): 534-539.
28.Kheradmand A, Alirezael M. and Birjandi M. 2010. Ghrelin promotes antioxidant enzyme activity and reduces lipid peroxidation in the rat ovary. Regul. Pept., 162: 84–9.
29.Kheradmand, A, Alirezael M, Asadian P, Alavi ER. and Joorabi S. 2009. Antioxidant enzyme activity and MDA level in the rat testis following chronic administration of ghrelin. Andrologia, 41: 335–40.
30.Kojima, M, Hosoda H. and Kangawa K. 2004. Ghrelin, a novel growth-hormone-releasing and appetite-stimulating peptide from stomach. Best Practice & Research Clinical Endocrinology & Metabolism, 18: 517–30.
31.Konturek, P, Brzozowski T, Walter B, Burnat G, Hess T, Hahn E. et al. 2006. Ghrelin induced gastroprotection against ischemia-reperfusion injury involves an activation of sensory afferent nerves and hyperemia mediated by nitric oxide. European Journal of Pharmacology, 536: 171–81.
32.Liu, L, Xu H, Jiang H, Wang J, Song N. and Xie J. 2010. Ghrelin prevents 1-methyl-4-phenylpyridinium ion induced cytotoxicity through antioxidation and NF-κB modulation in MES23.5 cells. Exp. Neurol., 222: 25–9.
33.Liu, M, Liang Y, Chigurupati S, Lathia JD, Pletnikov M, Sun Z, Crow M, Ross CA, Mattson MP and Rabb. H. 2008. Acute Kidney Injury Leads to Inflammation and Functional Changes in the Brain J Am Soc Nephrol. July; 19(7): 1360–1370.
34.Luck, H. Catalase. In: Bergmeyer H, editor. 1965. Methods of enzymatic analysis. Academic Press, New York, p. 855–84.
35.Obay, BD, Tasdemir E, Tumer C, Bilgin HM. and Atmaca M. 2008. Dose dependent effects of ghrelin on

pentylenetetrazole induced oxidative stress in a rat seizure model. Peptides; 29:448–55.
36.Paladino, J.D, Hotchkiss J.R. and Rabb H. 2009. Acute kidney injury and lung dysfunction: a paradigm for remote organ effects of kidney disease? Microvasc. Res., 77: 8–12. [PubMed: 18929580]
37.Park, SW, Chen SW, Kim M et al. 2011. Cytokines induce small intestine and liver injury after renal ischemia or nephrectomy. Lab Invest; 91: 63–84.
38.Rabb, H, Chamoun F. and Hotchkiss J. 2001. Molecular mechanisms underlying combined kidney-lung dysfunction during acute renal failure. Contrib. Nephrol., 41–52.
39.Rabb, H, Daniels F, O'Donnell M, Haq M, Saba S, Keane W. et al. 2000. Pathophysiological role of T lymphocytes in renal ischemia-reperfusion injury in mice. Am. J. Physiol. Renal. Physiol.; 279: 525-531.
40.Rajanc, D, Rongqian WU, Shah KG et al. 2012. Human ghrelin protects animals from renal ischemia-reperfusion injury through the vagus nerve. Surgery, 151: 37-47.
41.Sakon, M, Ariyoshi H, Umeshita K. and Monden M. 2002. Ischemia-reperfusion injury of the liver with special reference to calcium dependent mechanisms. Surg. Today; 32: 1–12.
42.Serteser, M, Koken T, Kahraman A, Yilmaz K, Akbulut G. and Dilek O. 2002. Changes in hepatic TNF-[alpha] levels, antioxidant status, and oxidation products after renal ischemia/reperfusion injury in mice. J. Surg. Res., 107: 234-240.
43.Shiiya, T, Nakazato M, Mizuta M, Date Y, Mondal M, Tanaka M. et al. 2002. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. Journal of Clinical Endocrinology & Metabolism; 87: 240-4.
44.Sumimoto, K, Oku J, Dohi K. and Kawasaki T. 1990. Lipid peroxidation in transplanted rat liver. Transplant Proc., 22: 2023–4.
45.Sun, Y, Oberley LW. and Li.Y, 1988. A simple method for clinical assay of superoxide dismutase, Clin. Chem., 34: 479–500.
46.Takeda, R, Nishimatsu H, Suzuki E, Satonaka H, et al. 2006. Ghrelin Improves Renal Function in Mice with Ischemic Acute Renal Failure. J. Am. Soc. Nephrol. 17: 113–121.
47.Takeda, R, Nishimatsu H, Suzuki E, Satonaka H, Nagata D, Oba S, Sata M. Takahashi M, Yamamoto Y, Terauchi Y, Kadowaki T, Kangawa K, Nagai R. and Hirata Y. 2006. Ghrelin improves renal function in mice with ischemic acuterenal failure. J. Am. Soc. Nephrol., 17: 113–21.
48.Tan, DX, Manchester LC, Sainz RM, Mayo JC, León J. and Reiter RJ. 2005. Physiological ischemia/reperfusion phenomena and their relation to endogenous melatonin production: a hypothesis. Endocrine. Jul; 27(2):149-58
49.Yassin, MM, Harkin DW, Barros D'Sa AA, Halliday MI. and Rowlands BJ. 2002. Lower limb ischemia-reperfusion injury triggers a systemic inflammatory response and multiple organ dysfunction. World J. Surg., 26: 115–21.
50.Yildirim, A, Gumus M, Dalga S. et al. 2003. Dehydroepiandrosterone improves hepatic antioxidant systems after renal ischemia-reperfusion injury in rabbits. Ann. Clin. Lab. Sci., 33: 459-464.
51.Yoshihara, F, Kojima M, Hosoda H, Nakazato M. and Kangawa K. 2002. Ghrelin: a novel peptide for growth hormone release and feeding regulation. Current Opinion in Clinical Nutrition & Metabolic Care, 5: 391–5.
52.Zwirska, K.K., Adamczyk, S.M, Sowa, P. et al. 2007. Role of leptin, ghrelin, angiotensin II and orexins in 3 T3 L1 preadipocyte cells proliferation and oxidative metabolism. J. Physiol. Pharmacol., 58: 53–64.