Original Article

Superior Protective Effects of Febuxostat Plus Alpha-Lipoic Acid on Renal Ischemia/Reperfusion-Induced Hepatorenal Injury in Rats

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ABSTRACT. A complex cascade of pathological events including oxidative stress and inflammation is involved in ischemia/reperfusion (I/R)-induced local and remote organ injuries. This study was performed to evaluate the effects of febuxostat (FEB), a selective xanthine oxidase (XO) inhibitor, and alpha-lipoic acid (ALA), a strong antioxidant, on the kidney and liver changes induced by renal I/R in rats. Renal I/R was induced in rats by clamping renal pedicles for 1 h followed by 2 h reperfusion. Fifty rats were assigned to five groups as follows: sham operated; vehicle + I/R; FEB + I/R; ALA + I/R, and (FEB + ALA) + I/R. Drug treatment was given 24 h and 1 h before I/R induction. Serum and tissue biochemical parameters and histopathological changes were examined after reperfusion. Serum creatinine, urea and uric acid levels, and alanine aminotransferase and aspartate aminotransferase activities were elevated after renal I/R. An increase in XO, myeloperoxidase, and malondialdehyde levels was observed in kidney and liver tissues with a concomitant decrease in both the glutathione level and superoxide dismutase activity. In addition, kidney and liver sections of vehicle-pretreated rats subjected to I/R exhibited a pronounced alteration in microanatomy. FEB, ALA, or FEB + ALA pretreatment attenuated the serum and tissue biochemical changes with amelioration of the histopathological changes in both the kidney and liver. The findings of this study revealed that FEB in combination with ALA had a greater protective effect than either drug alone. Thus, FEB and ALA co-administration may provide a potential superior therapeutic strategy to protect the kidney and liver against renal I/R-induced injury.

Introduction

Renal ischemia, whether a sequel to surgery, transplantation, or other causes, is a major cause of acute renal failure.¹ Renal cells have higher rate of baseline oxygen use by renal cells, rendering them incapable of increasing oxygen transport in response to hypoxia, thus leading to tubular cell injury.² Furthermore, evidence suggests that restoration of blood flow after ischemia may augment local tissue injury in excess of that produced by ischemia alone.³ In addition, injury to organs remote
from the site of ischemia has been observed following reperfusion of ischemic tissues, which suggests that circulating humoral and/or cellular mediators originating from ischemic tissues are responsible for mediating remote organ injuries. The pathophysiology of renal ischemia/reperfusion (I/R) injury includes multiple interrelated mechanisms. Importantly, studies have demonstrated that oxidative stress plays a major role in I/R-induced injury. During the I/R period, robust reactive oxygen species (ROS) generation beyond the protective abilities of endogenous antioxidants could result in oxidative damage to cellular biomolecules. In addition, I/R may initiate a local damaging inflammatory response characterized by pro-inflammatory cytokine induction and neutrophil infiltration. In addition, the presence of neutrophils in the ischemic region and their adhesion to vascular endothelial cells and infiltration into inflamed tissues after reperfusion contribute to the development of I/R-induced organ injury.

A substantial amount of evidence suggests that xanthine oxidase (XO) is a critical source of ROS production and oxidative stress in a variety of pathological conditions. In addition, studies with the XO inhibitor allopurinol have shown beneficial effects in animal models of I/R injury. Febuxostat (FEB), a nonpurine XO inhibitor with a favorable safety profile, has been reported to have a greater protective effect than allopurinol in these models. In addition, FEB reduces the production of uric acid which itself has been shown to induce oxidative stress. Alpha-lipoic acid (ALA) is a naturally occurring dithiol potent scavenger of free radicals. In the past few years, a growing interest has been given to its antioxidant effects, and there has been a marked rise in the number of publications confirming its potential therapeutic benefits in a variety of pathological states associated with pro-oxidant–anti-oxidant imbalance. Furthermore, in several in-vivo models, the co-administration of ALA with some other drugs has been shown to have a greater protective effect than either agent alone.

The aim of this study was to determine whether the co-administration of FEB and ALA would have better protective effects on the kidney and liver than the administration of either drug alone in a rat model of kidney damage induced by bilateral renal I/R. This objective was verified depending on measuring serum and tissue biochemical parameters and histopathological examination to evaluate kidney and liver injuries in the renal I/R model in rats.

Materials and Methods

Animals and drugs

The protocol of this study was approved by the Research Ethics Committee at the Medical Research Institute Alexandria University, and the principles of laboratory animal care were followed in all experiments. Fifty adult male albino rats weighing 200–250 g were included in this study. All rats were allowed free access to rat chow and water ad libitum and housed two per cage under standard environmental conditions (22°C–25°C, 12 h light/dark cycle).

FEB (Medizen Pharmaceutical Industries Co., Alexandria, Egypt) and ALA (Sigma Aldrich Chemical Co., St. Louis, USA) were dissolved in 5% (w/v) dimethylsulfoxide (DMSO) solution and administered in a constant volume of 1 mL. Fresh drug solutions were prepared at the beginning of each experiment. In all groups, drug (or vehicle) treatment was given at 24 h and 1 h before the surgical procedure.

Ischemia/reperfusion induction

Rats were fasted overnight with free access to water. Rats were anesthetized with pentobarbital sodium (30 mg/kg, i.p.). A median laparotomy was made to expose both kidneys. The blood supply to the kidneys was interrupted for 1 h by clamping both renal pedicles using a nontraumatic microvascular clamp. Renal ischemia was followed by 2 h of reperfusion achieved with the removal of the clamps. During the surgical procedure, the rat was positioned under a heating lamp to preserve the body temperature.
Animal grouping
The rats were divided into five groups of ten rats each: (1) sham-operated control group, treated with the vehicle (1 mL DMSO) for two days and, thereafter, subjected to a sham operation without clamping the renal pedicles; (2) I/R group, treated similarly with DMSO and, thereafter, subjected to the clamping followed reperfusion; (3) FEB + I/R group, treated with FEB at a dose of 10 mg/kg by oral gavage\textsuperscript{20} for two days before I/R; (4) ALA + I/R group, treated with ALA at a dose of 100 mg/kg, i.p.\textsuperscript{21} for two days before I/R; and (5) (FEB + ALA) + I/R group, treated with FEB and ALA as in Groups 3 and 4.

Sample collection
At the end of the reperfusion period, the rats were killed by exsanguination of the abdominal aorta. The blood sample, obtained from each rat, was left for 60 min to clot, and serum was separated by centrifugation and stored at \(-20^\circ\text{C}\) until analysis. Immediately after blood collection, the two kidneys and liver of each rat were removed. The upper halves of the right kidneys and samples of liver tissue taken from the right lobe were fixed in 10% phosphate-buffered formalin for at least three days before being processed for histopathological examination. The rest of the kidney and liver tissues were rinsed with ice-cold saline, blotted dry, and kept at \((-80^\circ\text{C})\) till used for tissue biochemical assessment.

Histological evaluation
After formalin fixation and dehydration, the tissue specimens were embedded in paraffin, and 4 \(\mu\text{m}\) sections were cut and stained with hematoxylin and eosin. Kidney and liver sections were examined under a light microscope for the histopathological changes by a pathologist who was unaware of the rat groups.

Estimation of serum biochemical parameters
Kidney function (serum creatinine and urea concentrations) and liver function (serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities) were determined using commercially available assay kits (Diamond Diagnostics, Cairo, Egypt) according to the manufacturer’s instructions. Serum uric acid concentration was determined with a kit from BioScope Diagnostics Co. (Cairo, Egypt).

Estimation of tissue myeloperoxidase and xanthine oxidase levels
Ten percent homogenates of kidney and liver tissue samples were prepared in phosphate-buffered saline (pH = 7.4) using a Potter–Elvehjem homogenizer. The homogenates were centrifuged at 3000 rpm for 20 min, and the supernatants were used for tissue assays. Myeloperoxidase (MPO) and XO tissue levels were quantified in the supernatant samples using rat MPO and XO enzyme-linked immunosorbent assay kits, respectively (Sunred Biotechnology, Shanghai, China) according to the manufacturer’s instructions.

Estimation of tissue reduced glutathione and malondialdehyde levels and superoxide dismutase activity
Homogenates of kidney and liver samples were prepared as described previously\textsuperscript{22} and used for the measurement of tissue oxidative stress markers. Glutathione (GSH) level was quantified by a colorimetric method based on the reduction of 5, 5′-dithiobis-2-nitrobenzoic acid with GSH.\textsuperscript{23} Tissue malondialdehyde, the main product of lipid peroxidation, was measured by a colorimetric method based on the reaction between MDA and thiobarbituric acid.\textsuperscript{24} Tissue superoxide dismutase (SOD) activity was determined by a method based on the inhibition of nitroblue tetrazolium reduction as described by Sun et al.\textsuperscript{25}

Data analysis
All experimental data were expressed as mean \(\pm\) standard error of the mean. Data analysis was performed using the computer package Statistical Package for the Social Sciences (SPSS) for Windows version 11.5 (SPSS Inc., Chicago, IL, USA). Data were analyzed by one-way analysis of variance, and Tukey’s test was applied for post hoc analysis. The correlation between variables was tested
by computing the correlation coefficient (r, Pearson’s test). \( P < 0.05 \) was considered statistically significant.

**Results**

**Effect of drug pretreatment on renal ischemia/reperfusion-induced changes in serum biochemical markers**

Serum creatinine and urea and uric acid levels in the vehicle-treated I/R group were significantly higher than those in sham-operated control rats (Table 1). These elevations were significantly ameliorated in rats treated with FEB, ALA, or FEB + ALA before renal I/R induction. The intensity of improvement produced by FEB alone or in combination with ALA was of a greater extent than that produced by ALA alone. Similarly, serum ALT and AST activities showed a significant increase in the vehicle-treated I/R group, and pretreatment with FEB, ALA, or FEB + ALA attenuated the increase in the serum activities of both enzymes. As shown in Table 1, the treatment with FEB + ALA was significantly more effective than treatment with FEB alone in ameliorating the elevation in serum ALT and AST activities.

**Effect of drug pretreatment on renal ischemia/reperfusion-induced changes in kidney and liver xanthine oxidase levels**

In kidney and liver tissues, XO levels significantly increased in the vehicle-treated I/R group, compared to that of the sham-operated control group (Figure 1A and B). Pretreatment with FEB or ALA significantly attenuated the increase in tissue XO levels. Statistically, the effect produced by FEB was greater than that produced by ALA, and normalization of the tissue XO level in both the kidney and liver was observed only in the (FEB + ALA)-pretreated I/R group.

**Effect of drug pretreatment on renal ischemia/reperfusion-induced changes in kidney and liver myeloperoxidase levels**

As shown in Figure 2, there was a striking elevation in kidney tissue MPO levels in the vehicle-treated I/R group, compared to that of the sham-operated control group. However, pretreatment of rats with FEB or ALA or FEB + ALA attenuated the increase in MPO level in kidney tissue. Normalization of renal tissue MPO levels was observed only in the (FEB + ALA) + I/R group (Figure 2A). Similarly, liver tissue MPO levels significantly increased in the vehicle-treated I/R group, compared to that of the sham-operated control group. Pretreatment with either FEB or ALA before I/R induction significantly attenuated the increase in hepatic MPO levels, whereas pretreatment with FEB + ALA prevented any significant increase in the tissue MPO level (Figure 2B).

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Table 1. Serum parameters in sham-operated control, ischemia/reperfusion and febuxostat-, alpha-lipoic acid-, and (febuxostat + alpha-lipoic acid)-pretreated ischemia/reperfusion groups.

| Experimental groups | Urea (mg/dL) | Creatinine (mg/dL) | Uric acid (mg/dL) | ALT (U/L) | AST (U/L) |
|---------------------|-------------|--------------------|-------------------|-----------|-----------|
| Sham operated       | 35.02±1.31  | 1.28±0.04          | 3.48±0.37         | 39.37±0.54| 40.53±0.76|
| I/R                 | 65.38±2.61a | 2.45±0.17a         | 10.78±0.51a       | 107.20±1.46a| 122.59±1.97a|
| FEB+I/R             | 44.18±1.24ab| 1.36±0.040b        | 3.90±0.33b        | 58.94±0.74b| 63.21±1.79ab|
| ALA+I/R             | 50.70±1.46ab| 1.66±0.07ab        | 5.05±0.23ab       | 49.69±1.45ab| 57.43±1.11ab|
| (FEB+ALA)+I/R       | 40.88±1.69xc| 1.31±0.02bc        | 3.62±0.16bc       | 45.61±2.44bc| 52.34±1.79bd|

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, I/R: Ischemia/reperfusion, FEB: Febuxostat, ALA: Alpha-lipoic acid.

Values shown are mean ± standard error of the mean (n = 10). \(^aP<0.05\) versus sham-operated group; \(^bP<0.05\) versus I/R group; \(^cP<0.05\) versus ALA + I/R group; \(^dP<0.05\) versus FEB+I/R group.
Figure 1. Kidney (A) and liver (B) tissue xanthine oxidase levels in sham-operated control, ischemia/reperfusion and febuxostat-, alpha-lipoic acid-, and (febuxostat + alpha-lipoic acid)-pretreated ischemia/reperfusion groups. Values shown are mean ± standard error of the mean, (n = 7–10 per group).

XO: Xanthine oxidase, FEB: Febuxostat, I/R: Ischemia/reperfusion, ALA: Alpha-lipoic acid.

*P < 0.05 versus sham-operated group, †P < 0.05 versus I/R group, ‡P < 0.05 versus FEB + I/R group, ¶P < 0.05 versus ALA + I/R group.

Figure 2. Myeloperoxidase levels in kidney (A) and liver (B) tissues of sham-operated control, ischemia/reperfusion and febuxostat-, alpha-lipoic acid-, and (febuxostat + alpha-lipoic acid)-pretreated ischemia/reperfusion groups. Values shown are mean ± standard error of the mean (n = 7–10 per group).

MPO: Myeloperoxidase, I/R: Ischemia/reperfusion, FEB: Febuxostat, ALA: Alpha-lipoic acid.

*P < 0.05 versus sham-operated group, †P < 0.05 versus I/R group, ‡P < 0.05 versus FEB + I/R group, ¶P < 0.05 versus ALA + I/R group.
resulted in significant changes in kidney and liver tissue oxidative stress markers, including an increase in tissue MDA levels and a decrease in both GSH levels and SOD activities. Administration of FEB, ALA, or FEB + ALA before renal I/R induction significantly attenuated the changes in these markers, in both organs, as compared to the vehicle-pretreated I/R group. The intensity of the attenuating effect produced by FEB + ALA, especially on kidney and liver MDA levels, was of a greater extent than that produced by either drug alone.

As shown in Table 3, the results from all experimental groups showed that serum indices of organ function and uric acid and tissue levels of MPO and XO correlated positively with the corresponding tissue MDA level and negatively with the antioxidant tissue markers (GSH level and SOD activity) in both the kidney and liver (Table 3).

**Histopathological findings**

Histological examination of kidney and liver tissue specimens obtained from sham-operated control rats showed the normal architecture of both organs (Figure 3a and f). In comparison with the sham-operated control group, the kidneys of vehicle-pretreated rats subjected to I/R showed areas with leukocyte infiltration, cast formation, tubular dilatation, and tubular cell necrosis (Figure 3b). The liver histopathological changes induced by renal I/R were more prominent in the periportal than the centrilobular regions of hepatic lobules and included irregularity of liver cell cords with cellular degenerative changes and leukocyte infiltration (Figure 3g). Treatment with FEB, ALA, or FEB + ALA before renal I/R induction markedly attenuated the histopathological changes in both the kidney (Figure 3c-e) and liver (Figure 3h-j) with overall preservation of normal organ architecture.

**Discussion**

To our knowledge, this study is the first research work describing the combined use of FEB, a selective XO inhibitor, and ALA, a strong antioxidant with free radical scavenging properties, to protect against renal I/R-induced local kidney and remote liver injuries. In rats subjected to renal I/R, in this study, local responses in the kidney and remote effects on the liver, including changes in function, morphology, inflammatory status, and oxidant–antioxidant balance, were observed in both organs. These changes were significantly attenuated in rats treated with FEB, ALA, or simultaneously with both drugs before renal I/R induction.

Although the pathophysiology of I/R-induced injury is complex, ROS generated during tissue I/R are well established as critical mediators of damage to cellular membranes or macromolecules. A main cause of oxidative stress during reperfusion of ischemic tissues is XO which transfers electrons to O2, producing ROS such as superoxide and hydroxyl radicals. Under ischemic conditions, adenosine triphosphate is broken down into hypoxanthine and xanthine which are finally oxidized to uric acid via XO. The low activity of this enzyme in nonischemic tissues suggests that this oxidation reaction is quite slow under normal conditions and cannot proceed during the period of ischemia due to the absence of oxygen. This missing substrate is supplied suddenly, and, to excess, at the moment of reperfusion with rapid overproduction of ROS which can induce oxidative stress. An excess of uric acid has also been found to induce oxidative stress in adipocytes and vascular endothelial and smooth muscle cells. In accordance with these findings, our results showed a significant increase in XO levels in the kidneys and livers of rats subjected to renal I/R. Furthermore, significant positive correlations between serum uric acid level and kidney and liver tissue levels of both XO and MDA, as an index of lipid peroxidation, were observed in this study.

An efficient endogenous antioxidant defense system is known to protect against tissue injury induced by free radicals. In our study, rats subjected to renal I/R showed a significant decrease in renal and hepatic tissue GSH levels and SOD activities, as indices of the
Table 2. Kidney and liver tissue levels of oxidative stress markers in sham-operated, ischemia/reperfusion and febuxostat-, alpha-lipoic acid-, and (febuxostat + alpha-lipoic acid)-pretreated ischemia/reperfusion groups.

| Experimental groups | Kidney tissue | Liver tissue |
|---------------------|--------------|-------------|
|                     | MDA (nmol/g) | GSH (nmol/g) | SOD (U/g) | MDA (nmol/g) | GSH (nmol/g) | SOD (U/g) |
| Sham-operated       | 47.48±1.54  | 5.92±0.13   | 505.79±5.01 | 27.28±1.30  | 9.70±0.24   | 880.29±3.33 |
| I/R                 | 94.08±1.76  | 2.52±0.09*  | 87.93±4.59  | 65.19±0.50*  | 8.37±0.16*  | 244.92±7.36* |
| FEB+I/R             | 59.39±0.65*  | 4.48±0.13*  | 476.77±4.85* | 42.78±0.82*  | 7.81±0.20*  | 799.75±23.81* |
| ALA+I/R             | 55.36±0.98*  | 4.81±0.14*  | 487.78±3.48  | 38.01±0.75*  | 8.41±0.24*  | 829.05±6.10* |
| (FEB+ALA) +I/R      | 50.17±0.67b,c,d | 5.49±0.16b,c,d | 493.52±4.81b | 32.13±0.59b,c,d | 9.07±0.13b,c | 868.10±2.82b,c |

MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase, I/R: Ischemia/reperfusion, FEB: Febuxostat, ALA: Alpha-lipoic acid. Values shown are mean±standard error of the mean (n = 10).

*P<0.05 versus sham-operated group; †P<0.05 versus I/R group; ‡P<0.05 versus FEB+I/R group; ‡‡P<0.05 versus ALA+I/R group.

Table 3. Correlation coefficients (r values) between the kidney and liver tissue oxidative stress markers and biochemical parameters using results from all experimental groups.

| Renal markers | Correlation coefficients (r) |
|---------------|------------------------------|
|               | Number of rats | MDA | GSH | SOD |
| Biochemical parameters |  |  |  |  |  |
| Serum urea    | 50             | 0.835* | −0.829* | −0.793* |
| Serum creatinine | 50              | 0.810* | −0.774* | −0.812* |
| Serum UA      | 50             | 0.913* | −0.840* | −0.924* |
| Renal tissue MPO | 45               | 0.958* | −0.880* | −0.967* |
| Renal tissue XO | 45              | 0.915* | −0.874* | −0.919* |
| Hepatic markers |  |  |  |  |  |
| Biochemical parameters |  |  |  |  |  |
| Serum ALT     | 50             | 0.952* | −0.949* | −0.961* |
| Serum AST     | 50             | 0.956* | −0.933* | −0.961* |
| Serum UA      | 50             | 0.875* | −0.878* | −0.933* |
| Hepatic tissue MPO | 45              | 0.946* | −0.939* | −0.972* |
| Hepatic tissue XO | 45              | 0.905* | −0.899* | −0.920* |

MDA: Malondialdehyde, GSH: Reduced glutathione, SOD: Superoxide dismutase, UA: Uric acid, MPO: Myeloperoxidase, XO: Xanthine oxidase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

*P <0.001.
endogenous antioxidant status, as compared to the sham-operated group. These decreases can disturb the balance between ROS production and natural antioxidant defenses and, subsequently, amplify ROS-induced tissue damage. 

Kidney and liver GSH levels and SOD activities were inversely correlated, in our study, with the serum parameters of kidney function (creatinine and urea levels) and liver function (ALT and AST activities), supporting the importance of endogenous antioxidants in providing protection against ROS-induced local and remote organ injury and dysfunction.

In addition, I/R has been shown to result in neutrophil activation, chemotaxis, adhesion to endothelial cells, and transmigration. Neutrophils produce MPO, proteases, cytokines, and ROS, leading to increased vascular permeability and reduced epithelial and endothelial cell integrity with a resultant increased ischemic injury. This is consistent with the histopathological findings and the significant increase in kidney and liver tissue levels of MPO, a marker of neutrophil recruitment, observed in the I/R group in this study, as compared to the control group. Furthermore, our results showed that, in both the kidney and liver, there were positive correlations between the tissue levels of MDA and MPO levels, confirming the recruitment of neutrophils in renal I/R-induced lipid peroxidation in both organs.

Improved understanding of the cellular and molecular mechanisms of I/R-induced tissue injury may enhance therapy. Accordingly, XO inhibition combined with scavenging free radicals seems to be a plausible therapeutic intervention to ameliorate I/R injury. In this study, our findings showed that treatment of rats with FEB, ALA, or with both drugs before renal I/R induction provided protection to the kidney and also to the liver, a commonly injured remote organ, as evidenced by several major findings. First, drug pretreatment significantly reduced the elevated serum
levels of urea and creatinine and ALT and AST activities. Second, drug pretreatment attenuated I/R-induced changes in kidney and liver MPO levels. Third, drug pretreatment, in I/R groups, reversed the changes in kidney and liver tissue XO, MDA, and GSH levels and SOD activity, compared to the vehicle-pretreated I/R group. Fourth, drug pretreatment markedly alleviated I/R-induced kidney and liver histopathological changes.

Thus, our findings implied that FEB was effective in providing protection against kidney and liver injuries in the renal I/R model. Several mechanisms may explain FEB protective effects in this model. One possible mechanism is the reduction of ROS generation and oxidative tissue damage via inhibiting XO activity. This is supported by our findings that rat pretreatment with FEB reduced renal I/R-induced oxidative stress in kidney and liver tissues. An additional mechanism is through the anti-apoptotic effect of FEB. Through its XO-inhibiting action with less ROS production, FEB can modulate ischemia-induced changes in mitochondrial membrane with less release into the cytoplasm of cytochrome C which activates caspases, enhances the expression of mitochondrial anti-apoptotic proteins, and decreases the expression of pro-apoptotic proteins, resulting in the suppression of apoptosis. In addition, the inhibition of uric acid production by FEB may have contributed to its protective effects observed in this study. As high intracellular uric acid levels can promote acute cellular inflammation and induce oxidative stress through the activation of nicotinamide adenine dinucleotide phosphate oxidase, FEB, via its inhibitory effect on XO, may halt the vicious circle involving intracellular uric acid and cell injury. Thus, the anti-inflammatory effect of FEB, observed in this study, may be explained, at least in part, by the less production of ROS and uric acid.

In addition, our study demonstrated that ALA pretreatment improved the morphology and function of both the kidney and liver in rats subjected to renal I/R. This hepatorenal protection may be attributed, at least in part, to ALA strong antioxidant effect. ALA and its metabolite, dihydrolipoic acid (DHLA), are capable of scavenging free radicals, have metal-chelating activity, and help regenerate endogenous antioxidants, such as vitamins C and E. Unlike other antioxidants, ALA and DHLA have both hydrophilic and lipophilic properties and, therefore, can cross biological membranes easily and exert their antioxidant action both in the cytosol and plasma membrane. Furthermore, ALA has been reported to induce de novo synthesis of GSH and inhibit apoptosis and inflammation, suggesting the contribution of additional mechanisms to its protective effect against I/R-induced tissue injury. In support of our results, ALA has been reported to reduce I/R-induced injury to various organs such as the intestine, ovary, liver, testis, and pancreas. In addition, there are reports that ALA can protect against kidney injury due to I/R. However, our study is the first reported work demonstrating the protective effect of ALA on the liver, as a remote organ, besides its nephroprotective effect following renal I/R injury.

In addition, it is worth mentioning that a major and perhaps more interesting finding, in this study, was that co-administration of FEB with ALA resulted in a greater significant reduction in tissue oxidative stress markers and inflammatory indicators with improvement in morphology and function of both the kidney and liver, as compared to I/R groups pretreated with either drug alone. Thus, it appears that FEB and ALA may interact, via their different mechanisms of action, to provide an additive protective effect locally in the kidney and remotely in the liver against renal I/R-induced injury.

In conclusion, our study suggests that therapy with the combination of FEB and ALA could provide a potential superior therapeutic strategy to attenuate hepatorenal inflammation, oxidative stress, and dysfunction that may occur as a sequel to renal I/R in some clinical settings such as kidney transplantation and major kidney surgery.
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