Ethyl pyruvate is renoprotective against ischemia-reperfusion injury under hyperglycemia

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ABSTRACT

Background: Hyperglycemia (HG) is common in cardiovascular surgeries due to diabetes, inflammation, and the neuroendocrine stress response. HG aggravates renal ischemia-reperfusion (I/R) injury through an increased inflammatory response, and blunts the protective effect of various measures. Ethyl pyruvate (EP) provides anti-inflammatory effects against I/R injury via inhibition of high-mobility group box 1 protein (HMGB1) release. This study aimed to determine the renoprotective effect of EP against I/R injury under HG.

Methods: Sprague–Dawley rats were randomly assigned at random to 8 groups: normoglycemia (NG)-sham, NG-I/R-control, NG-EP-I/R (pretreatment), NG-I/R-EP (posttreatment), HG-sham, HG-I/R-control, HG-EP-I/R, and HG-I/R-EP. Renal I/R was induced by 45 minutes of ischemia (clamping of renal arteries), followed by 24 hours of reperfusion. EP (50 mg/kg) was administered intraperitoneally at 1 h before ischemia (pretreatment) or on reperfusion (posttreatment).

Results: I/R injury under HG significantly aggravated the degree of renal tubular apoptosis and damage compared with the NG groups, which could be attenuated by both pretreatment and posttreatment of EP. I/R-induced increases in HMGB1 and Toll-like receptors (TLRs), activation of NF-κB, and resultant alterations in interleukin-1β, tumor necrosis factor-α, proapoptotic Bax, and antiapoptotic Bcl-2 were all favorably modulated by EP treatment in both the NG and HG groups compared with their corresponding control groups.

Conclusions: Despite aggravation of renal I/R injury by HG through amplified inflammation, EP administration showed similar suppression of the HMGB1-TLR-NF-κB pathway in the HG and NG groups. EP retained anti-inflammatory, antiapoptotic, and renoprotective effects in the HG groups, whether administered before ischemia or on reperfusion. (J Thorac Cardiovasc Surg 2018;155:1650-8)

EP significantly reduced apoptosis following renal I/R in normoglycemia and hyperglycemia.

Central Message

Ethyl pyruvate protects the kidneys against ischemia-reperfusion even under hyperglycemia whether administered before ischemia or on reperfusion.

Perspective

Hyperglycemia, which frequently accompanies cardiovascular operation, aggravates renal ischemia-reperfusion injury, and often mitigates protective measures. Ethyl pyruvate retains its protective effect even in hyperglycemia via preserved suppression of the HMGB1-TLR-NF-κB pathway. The proven safety of this compound enables rapid clinical translation.

See Editorial Commentary page 1659.

Cardiovascular surgeries continue to be complicated by acute kidney injury (AKI), which has been consistently identified as a portent of detrimental outcomes.¹ In that context, the

pathogenesis of perioperative AKI mainly involves ischemia/reperfusion (I/R) injury aggravated by the ensuing systemic inflammation.¹ Experimentally, several protective measures of AKI against I/R injury have been identified, including the commonly used anesthetics^{2,3}; however, most of these agents' beneficial effects on AKI after I/R injury are either annulled or attenuated by the presence of hyperglycemia (HG),⁴⁻⁶ which frequently accompanies cardiovascular surgeries irrespective of the presence of

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Abbreviations and Acronyms

AKI	= acute kidney injury
BUN	= blood urea nitrogen
EP	= ethyl pyruvate
HG	= hyperglycemia
HMGB1	= high-mobility group box 1 protein
IL	= interleukin
I/R	= ischemia/reperfusion
NG	= normoglycemia
TNF	= tumor necrosis factor
TLR	= Toll-like receptor

diabetes owing to increased inflammatory and neuroendocrine stress responses.^{7,8} HG is also known to intensify renal I/R injuries.⁹ Considering that the main mechanism of aggravated renal I/R injury under HG involves amplification of the inflammatory response,¹⁰ therapies aimed at ameliorating the foremost key trigger of the inflammatory cascade should retain preventive efficacy against renal I/R injury regardless of the glycemic condition.

High-mobility group box 1 protein (HMGB1) is a nonchromosomal nuclear protein that can be released passively from necrotic cells or actively secreted by immune cells.¹¹ Extracellular HMGB1 has been identified as the foremost critical inflammatory mediator in various pathological conditions, including I-R injury.^{12,13}

Ethyl pyruvate (EP), a simple aliphatic ester of the metabolic intermediate pyruvate, provides protective effect against organ damage through its anti-inflammatory effects in various conditions, including I/R injury.^{14,15} Moreover, previous studies have shown that EP exerts its protective effects through inhibition of HMGB1 release in models of inflammatory disease or renal I/R injury.^{16,17} EP also was shown to ameliorate renal dysfunction in an experimental model of diabetic nephropathy.¹⁸ Evidence regarding the effect of EP on renal I/R injury under HG conditions is lacking, however.

The present study was conducted to investigate whether the renoprotective effect of EP against I/R injury can be preserved under HG conditions and to elucidate the relevant mechanisms related to HMGB1, Toll-like receptor (TLR)-2, and TLR-4 in a rat model of renal I/R injury.

METHODS

Animal Preparation

All animal experiments were approved by the Committee for the Care and Use of Laboratory Animals at Yonsei University College of Medicine, and were performed in accordance with the US National Institutes of Health's Guide for the Care and Use of Laboratory Animals.¹⁸ Male Sprague-Dawley rats (10 to 12 weeks old, 250 to 300 g) were anesthetized with Rompun (10 mg/kg; Bayer DVM, Shawnee Mission, Kan) and Zoletil 50 (30 mg/kg; Virbac, Seoul, Korea). The rats were intubated with a 16-gauge catheter and artificially ventilated (model 683 small animal

ventilator; Harvard Apparatus, Holliston, Mass) at 30 to 35 cycles/minute. The right femoral artery was cannulated to monitor mean arterial pressure and obtain blood samples. Heart rate was monitored by subcutaneous stainless steel electrodes connected to the PowerLab data acquisition system (PowerLab ML845 with ML132; AD Instruments, Colorado Springs, Colo). Body temperature was continuously monitored and maintained around 37°C using a heating pad throughout the experiment.

Experimental Models and Study Groups

We used a well-established rat model of renal I/R injury in which the structural and functional consequences of brief periods of renal ischemia have been described.³ In brief, the rats underwent 45 minutes of global ischemia by clamping of both renal arteries with nontraumatic microvascular clamps, followed by 24 hours of reperfusion. I/R was confirmed by visual inspection of the kidney. The chosen duration of ischemia was based on our preliminary experiment showing the most consistent renal tubular injury without causing mortality. Mean arterial pressure and heart rate were continuously monitored and recorded throughout the procedure (ie, baseline, during ischemia, and after reperfusion). To examine the effect of EP on renal function, EP (50 mg/kg; total volume of 200 μ L; Sigma-Aldrich, St. Louis, Mo) was administered intraperitoneally at 1 hour before ischemia or on reperfusion, and the control group received an equivalent amount of normal saline at the same time points. The chosen dose of EP (50 mg/kg) in the present study was from previous studies investigating EP and I/R injury¹⁹ and our preliminary study, in which we tested the efficacy of various dosages from 30 mg/kg in 10-mg/kg increments and found that 50 mg/kg yielded the maximum renoprotective effects in terms of tubular damage, with no further benefit provided by a higher dosage. The rats in the HG groups received dextrose (1.2 g/kg) at 1 h before ischemia, whereas the normoglycemia (NG) groups received equivalent volume of normal saline via the tail vein. A blood sugar concentration >200 mg/dL was considered HG.⁹ Blood sugar concentrations were serially monitored at baseline, before ischemia, on reperfusion, and 24 hours thereafter.

The animals were assigned at random to 8 groups: NG-sham group (n = 9), normal saline only; NG-untreated control I/R group (n = 9), normal saline + I-R; NG-EP pretreatment I/R group (n = 9), normal saline + EP + I/R; NG-EP posttreatment I/R group (n = 9), normal saline + I/R + EP; HG-sham group (n = 9), dextrose only; HG-untreated control I/R group (n = 9), dextrose + I/R; HG-EP pretreatment I/R group (n = 9), dextrose + EP + I/R; and HG-EP posttreatment I/R group (n = 9), dextrose + I/R + EP. Kidneys were collected at the end of reperfusion.

Blood Urea Nitrogen and Creatinine Analysis

Blood urea nitrogen (BUN) and serum creatinine concentrations were determined at 24 hours after reperfusion using the picric acid and diacetyl monoxime methods, respectively.

Preparation of Kidney Tissue

The left kidneys were excised and sliced into 2 coronal sections. One piece was snap-frozen and stored at -80°C for immunoblot analysis, whereas the other piece was fixed in 10% formaldehyde and paraffin-embedded for histopathology examination, terminal deoxynucleotidyl transferase-mediated uridine triphosphate nick-end labeling (TUNEL) assay, and immunofluorescence. Kidney tissue preparations were cross-sectioned at a thickness of 5 μ m.

Histopathology Examination

Kidney tissues were obtained at the end of the 24-hour reperfusion. Periodic acid Schiff staining was performed as described previously.²⁰ The degree of tubular damage was graded on a scale from 0 to 3 (0 = no tubular necrosis; 1 = focal area of tubular necrosis involving <25% of the kidney; 2 = tubular necrosis involving 25% to 50% of the kidney; 3 = tubular

TABLE 1. Time course of blood glucose concentrations

Group	Baseline (n = 9), mg/dL	Before ischemia (n = 9), mg/dL	End of ischemia (n = 9), mg/dL	At 24 h after reperfusion (n = 9), mg/dL
Normoglycemia				
Sham	126 ± 20	121 ± 13	137 ± 14	144 ± 29
Control	121 ± 14	146 ± 27	122 ± 14	142.2 ± 13
Pre-EP	139 ± 13	142 ± 8	130 ± 9	139 ± 14
Post-EP	124 ± 10	136 ± 12	130 ± 13	135 ± 17
Hyperglycemia				
Sham	130 ± 16	399 ± 72*,†	458 ± 82*,†	244 ± 72*
Control	122 ± 14	311 ± 55*,†	324 ± 59*,†	250 ± 68*
Pre-EP	126 ± 14	358 ± 62*,†	428 ± 76*,†	263 ± 53*
Post-EP	124 ± 10	345 ± 88*,†	408 ± 82*,†	258 ± 69*

Data are mean ± SD. *Pre-EP*, Pretreatment of ethyl pyruvate at 1 hour before ischemia; *Post-EP*, posttreatment of ethyl pyruvate on reperfusion. **P* < .05 compared with baseline. †*P* < .05 compared with the normoglycemia groups.

necrosis involving >50% of the kidney) by a renal pathologist who was blinded to study group.³

TUNEL Assay

Detection of apoptosis in paraffin sections was performed using the TUNEL assay as described previously.²¹ The kidney tissue sections were stained using the in situ DeadEnd Colorimetric Apoptosis Detection System (Promega, Madison, Wisc) according to the manufacturer's instructions. Five visual fields from each sample block were selected at random and analyzed by a blinded observer using an Olympus microscope at a magnification of 400×. The apoptotic index was analyzed

(apoptotic cells/total cells × 100%) from a total of 20 fields per sample.

Immunofluorescence

Immunofluorescence staining was examined for TLR-2 and TLR-4. Tissue sections were stained with rabbit anti-TLR-2 and anti-TLR-4, followed by staining with FITC-conjugated secondary antibody (all Santa Cruz Biotechnology, Calif). Slides were viewed with an Olympus U-LH100HG fluorescence microscope (Olympus America, NY) equipped with an Olympus DP71 digital camera (20×).

Immunoblot Analysis

After appropriate treatments, equal amounts of protein from each group were subjected to immunoblot assay as described previously.³ Proteins were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotted with anti-HMGB1 (Abcam, Cambridge, Mass), anti-TLR-2, anti-TLR-4 (Santa Cruz Biotechnology), anti-Bcl-2, anti-Bax, anti-phospho-NF-κB, anti-NF-κB, and anti-GAPDH (all Cell Signaling Technology, Beverly, Mass).

ELISA

Levels of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in the serum were determined by ELISA commercial kits (R&D Systems, Minneapolis, Minn) according to the manufacturer's instructions.

Statistical Analysis

Data are expressed as mean ± SD or median (interquartile range). Hemodynamic data and serum glucose concentrations were analyzed by repeated-measures analysis of variance. Other variables were analyzed by 2-way analysis of variance except for the degree of tubular damage, which was analyzed by the Mann-Whitney *U* test. The *P* values of post hoc tests were adjusted by Bonferroni's methods. A *P* value < .05 was considered statistically significant.

RESULTS

Hemodynamic and Glycemic Parameters

Administration of dextrose increased blood glucose concentrations to >200 mg/dL in all rats in the HG groups before ischemia and on reperfusion. Glucose concentrations in the HG group remained significantly higher than their corresponding baseline values even at 24 hours after

TABLE 2. Hemodynamic data

Parameter	Baseline (n = 9)	End of ischemia (n = 9)	At 24 h after reperfusion (n = 9)
Mean arterial pressure, mmHg, mean ± SD			
Normoglycemia			
Sham	87 ± 18	88 ± 22	87 ± 20
Control	89 ± 21	88 ± 20	90 ± 6
Pre-EP	87 ± 12	92 ± 13	93 ± 21
Post-EP	86 ± 13	90 ± 12	95 ± 20
Hyperglycemia			
Sham	90 ± 10	87 ± 18	94 ± 17
Control	95 ± 9	90 ± 22	99 ± 15
Pre-EP	95 ± 15	94 ± 17	97 ± 11
Post-EP	92 ± 13	95 ± 16	98 ± 15
Heart rate, beats/min, mean ± SD			
Normoglycemia			
Sham	289 ± 22	294 ± 16	292 ± 16
Control	301 ± 18	300 ± 20	289 ± 15
Pre-EP	298 ± 34	310 ± 54	304 ± 34
Post-EP	290 ± 25	301 ± 29	300 ± 29
Hyperglycemia			
Sham	304 ± 24	300 ± 32	305 ± 29
Control	315 ± 49	310 ± 52	303 ± 59
Pre-EP	310 ± 41	319 ± 48	319 ± 57
Post-EP	311 ± 39	302 ± 33	310 ± 43

SD, Standard deviation; *Pre-EP*, pretreatment of ethyl pyruvate at 1 hour before ischemia; *Post-EP*, posttreatment of ethyl pyruvate on reperfusion.

FIGURE 1. Renal tubular apoptotic cell death assessed with terminal deoxynucleotidyl transferase-mediated uridine triphosphate nick-end labeling (A) and the degree of tubular damage (B) assessed using a scale of 0 to 3 (0 = no tubular necrosis, 1 = focal area of tubular necrosis involving <25% of the kidney, 2 = tubular necrosis involving 25% to 50% of the kidney, 3 = tubular necrosis involving >50% of the kidney) at 24 hours after ischemia-reperfusion. Control, Ischemia (45 minutes)-reperfusion (24 hours) without treatment; Pre-EP, pretreatment of ethyl pyruvate at 1 hour before ischemia; Post-EP, posttreatment of ethyl pyruvate on reperfusion; NG, normoglycemia; HG, hyperglycemia. $^{\dagger}P < .05$ versus sham within NG or HG; $^{\ddagger}P < .05$ versus control within NG or HG; $^{\S}P < .05$ versus corresponding group in NG (n = 9 each).

reperfusion (Table 1). Mean arterial pressure and heart rate were similar across the groups throughout the study period (Table 2).

EP Attenuates Renal Tubular Apoptotic Cell Death and Tubular Damage

I/R injury significantly increased the number of apoptotic tubular cells (Figure 1, A) and the degree of tubular damage (Figure 1, B) in both the NG and HG groups compared with their corresponding sham groups, whereas the former was significantly higher and the latter was significantly greater in the HG groups compared with their corresponding NG groups ($P < .05$). Both pretreatment and posttreatment, EP significantly reduced apoptotic cell death and the degree of tubular damage in the NG and HG groups compared with their corresponding control groups ($P < .05$), without any between-group (pretreatment vs posttreatment) differences (Figure 1, A and B).

EP Attenuates Increases in Serum Concentrations of BUN and Creatinine

Serum BUN and creatinine concentrations are shown in Figure 2. BUN and creatinine levels were increased by 24 hours after I/R in both the NG and HG groups compared with their corresponding sham groups, whereas BUN levels

were significantly higher in the HG group compared with the NG group ($P < .05$). Both pretreatment and posttreatment EP significantly ameliorated the increases in BUN and creatinine levels in the NG and HG groups compared with their corresponding control groups ($P < .05$), with no intergroup (pretreatment vs posttreatment) differences (Figure 2).

EP Attenuates Enhancement of HMGB1, TLR-2, and TLR-4 Expression and NF- κ B Phosphorylation

I/R induced changes of HMGB1, TLR-2, and TLR-4 expression (Figure 3) and NF- κ B phosphorylation (Figure 4) in kidney tissue under NG and HG conditions, and the effects of EP treatment on these parameters are shown in the figures. HG alone, without I/R injury, caused significant increases in HMGB1, TLR-2, and TLR-4 levels compared with the NG sham group ($P < .05$), but no changes in NF- κ B phosphorylation. I/R injury resulted in further significant increase in HMGB1, TLR-2, and TLR-4 levels and NF- κ B phosphorylation compared to their corresponding sham groups ($P < .05$). Both pretreatment and posttreatment EP significantly attenuated the increases in HMGB1, TLR-2, and TLR-4 levels and NF- κ B phosphorylation in the NG and HG groups compared with their corresponding control groups ($P < .05$), without any intergroup

FIGURE 2. Blood urea nitrogen (A) and serum creatinine (B) concentrations measured at 24 h after renal ischemia-reperfusion. *Control*, Ischemia (45 minutes)-reperfusion (24 hours) without treatment; *Pre-EP*, pretreatment of ethyl pyruvate at 1 hour before ischemia; *Post-EP*, posttreatment of ethyl pyruvate on reperfusion; *NG*, normoglycemia; *HG*, hyperglycemia. † $P < .05$ versus sham within NG or HG; ‡ $P < .05$ versus control within NG or HG; § $P < .05$ versus corresponding group in NG ($n = 9$ each).

(pretreatment vs posttreatment) differences ($P < .05$). All of these parameters remained significantly higher in the HG groups compared with the NG groups after EP treatment ($P < .05$).

EP Attenuates Increases in Serum Concentrations of TNF- α and IL-1 β

The effects of EP on serum concentrations of TNF- α and IL-1 β are shown in Figure 5, A. Following I/R, serum concentrations of TNF- α and IL-1 β were significantly increased in the NG and HG groups compared with their corresponding sham groups ($P < .05$), whereas only TNF- α was significantly higher in the HG group compared

with the NG group ($P < .05$). Both pretreatment and posttreatment of EP significantly mitigated the increased serum concentrations of TNF- α and IL-1 β ($P < .05$), with no intergroup (pretreatment vs posttreatment) differences.

EP Regulates I/R-Induced Alterations of Bax and Bcl-2 in HG

Figure 5, B, shows alterations of Bax and Bcl-2 and the effect of EP on these proteins under NG and HG. HG alone, without the I/R injury, caused a significant increase in proapoptotic Bax expression and a decrease in antiapoptotic Bcl-2 expression compared with the NG sham group

FIGURE 3. Immunoblot assays of the protein levels of HMGB1, TLR-2, and TLR-4 (A) and immunofluorescence images of TLR-2 (B) and TLR-4 (C) performed at 24 hours after renal ischemia-reperfusion. *NG*, Normoglycemia; *HG*, hyperglycemia; *EP*, ethyl pyruvate; *S*, sham; *I/R* (control), ischemia (45 minutes)-reperfusion (24 hours) without treatment; *Pre-EP*, pretreatment of ethyl pyruvate at 1 hour before ischemia; *Post-EP*, posttreatment of ethyl pyruvate on reperfusion; *HMGB1*, high-mobility group box 1; *TLR*, Toll-like receptor; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase. † $P < .05$ versus sham within NG or HG; ‡ $P < .05$ versus control within NG or HG; § $P < .05$ versus corresponding group in NG ($n = 9$ each).

FIGURE 4. Immunoblot assays of the protein levels of NF- κ B phosphorylation performed at 24 hours after renal ischemia-reperfusion. NG, Normoglycemia; HG, hyperglycemia; EP, ethyl pyruvate; S, sham; I/R (control), ischemia (45 minutes)-reperfusion (24 hours) without treatment; Pre-EP, pretreatment of ethyl pyruvate at 1 hour before ischemia; Post-EP, posttreatment of ethyl pyruvate on reperfusion; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. † $P < .05$ versus sham within NG or HG; ‡ $P < .05$ versus control within NG or HG; § $P < .05$ versus corresponding group in NG (n = 9 each).

($P < .05$). I/R injury resulted in further significant increases in Bax and decreases in Bcl-2 expression compared with their corresponding sham groups ($P < .05$). Both pretreatment and posttreatment EP significantly attenuated the increases in Bax expression and decreases in Bcl-2 expression in the NG and HG groups compared with their corresponding control groups ($P < .05$), with no intergroup (pretreatment vs posttreatment) differences (Figure 5, B). After EP treatment, Bax and Bcl-2 expression remained significantly higher and lower, respectively,

compared with the levels in their corresponding NG groups ($P < .05$).

DISCUSSION

In the present study, we have verified that HG aggravates renal I/R injury compared with NG in terms of apoptosis, tubular damage, and increased serum creatinine levels via exacerbation of HMGB1-TLR-NF- κ B-mediated inflammation. Administration of EP, either before ischemia or on reperfusion, could significantly attenuate the renal injury

FIGURE 5. Serum concentrations of tumor necrosis factor- α and interleukin-1 β assessed by ELISA (A), and immunoblot assays of the protein levels of proapoptotic Bax and antiapoptotic Bcl-2 (B) performed at 24 hours after renal ischemia-reperfusion. TNF, Tumor necrosis factor; NG, normoglycemia; HG, hyperglycemia; EP, ethyl pyruvate; S, sham; I/R (control), ischemia (45 minutes)-reperfusion (24 hours) without treatment; Pre-EP, pretreatment of ethyl pyruvate at 1 hour before ischemia; Post-EP, posttreatment of ethyl pyruvate on reperfusion; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. † $P < .05$ versus sham within NG or HG; ‡ $P < .05$ versus control within NG or HG; § $P < .05$ versus the corresponding group in NG (n = 9 each).

following I/R via favorable modulations of the accompanying inflammatory cascade, resulting in decreased proinflammatory cytokines, decreased TNF- α and IL-1 β expression, and up-regulation of antiapoptotic protein Bcl-2 expression, even in the HG condition.

Anatomically, the renal medulla has a distinctive vasculature that provides for medullary hypoxia to facilitate urinary concentration. Not surprisingly, AKI remains one of the most common complications following cardiovascular surgeries linked with unfavorable outcomes.¹ Although the pathogenesis of AKI after cardiovascular operations is complex, I/R and systemic inflammation are predominant, especially in operations involving the thoracoabdominal aorta, which are also frequently accompanied by HG. Perioperative HG is common not only in patients with diabetes, but also in patients without diabetes due to an increased inflammatory and neuroendocrine stress response.^{7,8} Irrespective of the presence of diabetes, HG is known to aggravate renal I/R injury through increased oxidative stress and an exacerbated inflammatory response,^{9,10} and to mitigate the effects of potentially protective measures.^{4,5} In conjunction, HG-induced worsening of renal function also has been observed in both critically ill and surgical patients,^{22,23} whereas strict glycemic control yielded disappointing results in terms of outcomes.²⁴ Thus, the development of an efficacious protective strategy against renal I/R injury under HG is a high priority for improving outcomes after cardiovascular surgeries.

HMGB1, constitutionally a DNA chaperone, has gained wide attention as a crucial mediator of the inflammatory response and I/R injury.¹³ When released either passively from necrotic cells or actively by secretion from inflammatory cells, extracellular HMGB1 acts as an endogenous danger-signaling molecule and mediates inflammation by binding to TLRs and activating the downstream signaling pathways, predominantly via NF- κ B.²⁵ In many I/R-induced organ injury models, HMGB1 has been proposed as the key molecule linking the initial injury from energy deprivation and the subsequent inflammatory response that causes further organ damage.¹³ Increased expression of HMGB1 has been previously reported in cells cultured with HG medium,²⁵ and HG rats have shown increased serum concentrations of HMGB1.²⁶ Thus, we hypothesized that inhibition of HMGB1 expression might retain its renoprotective effect against I/R injury, even when aggravated by HG through intensified inflammation.

Pyruvate, a metabolic intermediate, has a scavenging activity for reactive oxygen species.²⁷ EP, an ester formed from pyruvic acid and ethanol, is more stable in aqueous solution than pyruvate.¹⁵ Since Ulloa and colleagues¹⁶ reported EP's substantial anti-inflammatory effect via inhibition of HMGB1 release, many subsequent studies have demonstrated its protective effect in inflammatory disorders²⁸ and myocardial and renal I/R injury.^{17,19} EP also

has been shown to be protective against sepsis- or cisplatin-induced renal injury,^{29,30} and has been found to alleviate albuminuria and glomerular injury in experimental models of diabetic nephropathy.¹⁸ The mode of molecular action of EP involves anti-inflammatory and antiapoptotic action from down-regulating the HMGB1-TLR-NF- κ B axis, decreased autophagy through the competitive interaction of HMGB1 with Beclin1,³¹ and reduced oxidative stress.³² As mentioned above, EP's ability to inhibit the expression of HMGB1 could ameliorate renal I/R injury under HG, which has not been investigated previously. Above all, the clinically proven safety of EP³² would enable prompt clinical translation.

In accordance with previous studies, our results confirm that HG aggravates the renal I/R injury compared with NG in terms of tubular damage and apoptosis, as also reflected by an increase in serum creatinine concentration. We also verified that HG-induced aggravation of renal I/R injury is associated with increased HMGB1 expression, leading to enhanced activity of downstream TLR-2, TLR-4, and NF- κ B and resulting in an amplified inflammatory response. Of note, HG alone caused significant increases in HMGB1, TLR-2, TLR-4, and proapoptotic Bax, and a decrease in antiapoptotic Bcl-2 compared with NG. These results clearly implicate that acute HG, regardless of the presence of a long-standing diabetes, raises the inflammatory tone via HMGB1 even before the ischemic insult, resulting in aggravation of the ensuing renal I/R injury.

More importantly, we have demonstrated that EP can ameliorate I/R-induced kidney injury in association with attenuated expression of HMGB1-TLR-NF- κ B axis. As our results indicate, EP's inhibitory action on HMGB1 resulted in concomitant suppression of proinflammatory cytokines (TNF- α , IL-1 β), and favorable modulations of proapoptotic Bax and antiapoptotic Bcl-2, which are well-known key mediators of renal I/R injury.^{33,34} These beneficial influences of EP lead to lesser degrees of renal tubular damage and apoptotic cell death under both NG and HG conditions. Notably, the protective efficacy of EP appears to be well preserved under the HG condition, whereas protective measures against I/R injury reportedly are ineffective under HG.⁴⁻⁶ The ability of EP to retain its protective effect under HG may be explained by the fact that EP's action targets the common key pathogenic factor in both HG- and I/R-induced injury: expression of HMGB1,¹² which we have shown is increased by HG alone. Furthermore, EP treatment after I/R injury can provide a similar degree of renal protection, with a similar influence on the assessed pathways compared with preemptive administration. Therefore, the strength of the present study is that it not only provides primary evidence regarding the retained renoprotective efficacy of EP under HG against I/R injury, but also shows that these protective effects have

a large therapeutic window in relation to the onset of ischemic insult.

Requirements for pharmacologic treatment of patients at risk for renal I/R injury under moderate HG are common in clinical fields. Considering the clinically proven safety of EP,³² our present results should be of meaningful clinical significance in that regard, meriting further translational clinical studies.

This study has several limitations. First, although we applied an acute HG model on renal I/R injury to increase the clinical relevance of our study, our results cannot be extrapolated to a diabetes model. In this study, serum glucose concentrations after the administration of dextrose ranged from 200 to 400 mg/dL, a range commonly encountered in cardiovascular operations. Moreover, this range of glucose concentrations has been previously shown to aggravate renal I/R injury¹⁰ and render several protective measures ineffective.^{4,6} However, in cases accompanied by diabetes, receptors for advanced glycation end products also may play an important role, which has also been shown to be closely linked to HMGB1 in models of diabetic complications.³⁵ Evaluating this possibility is beyond the scope of the present study, requiring a different animal model for validation. Second, our renal I/R model cannot comprehensively simulate the AKI associated with cardiopulmonary bypass, which involves more complex pathophysiology. Our model may be more relevant to thoracoabdominal aortic operations; however, the chosen duration of ischemia (45 minutes) is unlikely to occur during surgery. In addition, tested rats are healthy and young, whereas many patients present with comorbidities that may affect the clinical course of AKI.

In conclusion, HG aggravates renal I/R injury through a more prominent increase in HMGB1 and amplified inflammation compared with NG. EP administration, either before or after the onset of I/R, could protect the kidneys from I/R injury even under HG through retained suppression of HMGB1 and its downstream inflammatory and apoptotic pathways.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

References

- O'Neal JB, Shaw AD, Billings FT IV. Acute kidney injury following cardiac surgery: current understanding and future directions. *Crit Care*. 2016;20:187.
- Lee HT, Kim M, Kim M, Kim N, Billings FT IV, D'Agati VD, et al. Isoflurane protects against renal ischemia and reperfusion injury and modulates leukocyte infiltration in mice. *Am J Physiol Renal Physiol*. 2007;293:F713-22.
- Yoo YC, Yoo KJ, Lim BJ, Jun JH, Shim JK, Kwak YL. Propofol attenuates renal ischemia-reperfusion injury aggravated by hyperglycemia. *J Surg Res*. 2013;183:783-91.
- Wang H, Chen H, Wang L, Liu L, Wang M, Liu X. Acute hyperglycemia prevents dexmedetomidine-induced preconditioning against renal ischemia-reperfusion injury. *Acta Cir Bras*. 2014;29:812-8.
- Schenning KJ, Anderson S, Alkayed NJ, Hutchens MP. Hyperglycemia abolishes the protective effect of ischemic preconditioning in glomerular endothelial cells in vitro. *Physiol Rep*. 2015;3:e12346.
- de Souza AV, Golim MA, Deffune E, Domingues MA, de Carvalho LR, Vianna IG, et al. Evaluation of renal protection from high doses of melatonin in an experimental model of renal ischemia and reperfusion in hyperglycemic rats. *Transplant Proc*. 2014;46:1591-3.
- Akhtar S, Barash PG, Inzucchi SE. Scientific principles and clinical implications of perioperative glucose regulation and control. *Anesth Analg*. 2010;110:478-97.
- Garg R, Grover A, McGurk S, Rawn JD. Predictors of hyperglycemia after cardiac surgery in nondiabetic patients. *J Thorac Cardiovasc Surg*. 2013;145:1083-7.
- Podrazik RM, Natale JE, Zelenock GB, D'Alecy LG. Hyperglycemia exacerbates and insulin fails to protect in acute renal ischemia in the rat. *J Surg Res*. 1989;46:572-8.
- Hirose R, Xu F, Dang K, Liu T, Behrends M, Brakeman PR, et al. Transient hyperglycemia affects the extent of ischemia-reperfusion-induced renal injury in rats. *Anesthesiology*. 2008;108:402-14.
- Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature*. 2002;418:191-5.
- Clune JR, Dhupar R, Cardinal J, Billiar TR, Tsung A. HMGB1: endogenous danger signaling. *Mol Med*. 2008;14:476-84.
- Tang D, Kang R, Zeh HJ III, Lotze MT. High-mobility group box 1, oxidative stress, and disease. *Antioxid Redox Signal*. 2011;14:1315-35.
- Sims CA, Wattanasirichaigoon S, Menconi MJ, Ajami AM, Fink MP. Ringer's ethyl pyruvate solution ameliorates ischemia/reperfusion-induced intestinal mucosal injury in rats. *Crit Care Med*. 2001;29:1513-8.
- Fink MP. Ethyl pyruvate: a novel anti-inflammatory agent. *J Intern Med*. 2007;261:349-62.
- Ulloa L, Ochani M, Yang H, Tanovic M, Halperin D, Yang R, et al. Ethyl pyruvate prevents lethality in mice with established lethal sepsis and systemic inflammation. *Proc Natl Acad Sci U S A*. 2002;99:12351-6.
- Rabadi MM, Ghaly T, Goligorsky MS, Ratliff BB. HMGB1 in renal ischemic injury. *Am J Physiol Renal Physiol*. 2012;303:F873-85.
- Ju KD, Shin EK, Cho EJ, Yoon HB, Kim HS, Kim H, et al. Ethyl pyruvate ameliorates albuminuria and glomerular injury in the animal model of diabetic nephropathy. *Am J Physiol Renal Physiol*. 2012;302:F606-13.
- Lin Y, Chen L, Li W, Fang J. Role of high-mobility group box-1 in myocardial ischemia/reperfusion injury and the effect of ethyl pyruvate. *Exp Ther Med*. 2015;9:1537-41.
- Choi DE, Jeong JY, Lim BJ, Chung S, Chang YK, Lee SJ, et al. Pretreatment of sildenafil attenuates ischemia-reperfusion renal injury in rats. *Am J Physiol Renal Physiol*. 2009;297:F362-70.
- Wang Y, Zhang ZZ, Wu Y, Ke JJ, He XH, Wang YL. Quercetin preconditioning attenuates myocardial ischemia/reperfusion injury in rats through the PI3K/Akt pathway. *Braz J Med Biol Res*. 2013;46:861-7.
- Fiaccadori E, Sabatino A, Morabito S, Bozzoli L, Donadio C, Maggiore U, et al. Hyper/hypoglycemia and acute kidney injury in critically ill patients. *Clin Nutr*. 2016;35:317-21.
- Mendez CE, Der Mesropian PJ, Mathew RO, Slawski B. Hyperglycemia and acute kidney injury during the perioperative period. *Curr Diab Rep*. 2016;16:10.
- Preiser JC, Devos P, Ruiz-Santana S, Mélot C, Annane D, Groeneveld J, et al. A prospective randomised multi-centre controlled trial on tight glucose control by intensive insulin therapy in adult intensive care units: the Glucontrol study. *Intensive Care Med*. 2009;35:1738-48.
- Yao D, Brownlee M. Hyperglycemia-induced reactive oxygen species increase expression of the receptor for advanced glycation end products (RAGE) and RAGE ligands. *Diabetes*. 2010;59:249-55.
- Tsuruta R, Fujita M, Ono T, Koda Y, Koga Y, Yamamoto T, et al. Hyperglycemia enhances excessive superoxide anion radical generation, oxidative stress, early inflammation, and endothelial injury in forebrain ischemia/reperfusion rats. *Brain Res*. 2010;1309:155-63.
- Kao KK, Fink MP. The biochemical basis for the anti-inflammatory and cytoprotective actions of ethyl pyruvate and related compounds. *Biochem Pharmacol*. 2010;80:151-9.
- Davé SH, Tilstra JS, Matsuoka K, Li F, DeMarco RA, Beer-Stolz D, et al. Ethyl pyruvate decreases HMGB1 release and ameliorates murine colitis. *J Leukoc Biol*. 2009;86:633-43.

29. Miyaji T, Hu X, Yuen PS, Muramatsu Y, Iyer S, Hewitt SM, et al. Ethyl pyruvate decreases sepsis-induced acute renal failure and multiple organ damage in aged mice. *Kidney Int.* 2003;64:1620-31.
30. Kelle I, Akkoc H, Tunik S, Nergiz Y, Erdinc M, Erdinc L. Protective effects of ethyl pyruvate in cisplatin-induced nephrotoxicity. *Biotechnol Biotechnol Equip.* 2014;28:674-80.
31. Shen M, Lu J, Dai W, Wang F, Xu L, Chen K, et al. Ethyl pyruvate ameliorates hepatic ischemia-reperfusion injury by inhibiting intrinsic pathway of apoptosis and autophagy. *Mediators Inflamm.* 2013;2013:461536.
32. Hu X, Cui B, Zhou X, Xu C, Lu Z, Jiang H. Ethyl pyruvate reduces myocardial ischemia and reperfusion injury by inhibiting high mobility group box 1 protein in rats. *Mol Biol Rep.* 2012;39:227-31.
33. Sharples EJ, Patel N, Brown P, Stewart K, Mota-Philipe H, Sheaff M, et al. Erythropoietin protects the kidney against the injury and dysfunction caused by ischemia-reperfusion. *J Am Soc Nephrol.* 2004;15:2115-24.
34. Wu H, Chen G, Wyburn KR, Yin J, Bertolino P, Eris JM, et al. TLR4 activation mediates kidney ischemia/reperfusion injury. *J Clin Invest.* 2007;117:2847-59.
35. Nogueira-Machado JA, Volpe CM, Veloso CA, Chaves MM. HMGB1, TLR and RAGE: a functional tripod that leads to diabetic inflammation. *Expert Opin Ther Targets.* 2011;15:1023-35.

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