Glutamine administration ameliorates sepsis-induced kidney injury by downregulating the high-mobility group box protein-1-mediated pathway in mice

Ya-Mei Hu,1 Man-Hui Pai,2 Chiu-Li Yeh,3 Yu-Chen Hou,1 and Sung-Ling Yeh1

1School of Nutrition and Health Sciences, 2Department of Anatomy, Taipei Medical University, and 3Department of Food and Nutrition, Chinese Culture University, Taipei, Taiwan

Submitted 6 May 2011; accepted in final form 8 September 2011

Hu YM, Pai MH, Yeh CL, Hou YC, Yeh SL. Glutamine administration ameliorates sepsis-induced kidney injury by downregulating the high-mobility group box protein-1-mediated pathway in mice. Am J Physiol Renal Physiol 302: F150–F158, 2012. First published September 14, 2011; doi:10.1152/ajprenal.00246.2011.—Acute kidney injury (AKI) is a severe complication of sepsis. High-mobility group box (HMGB)-1 was implicated as a late mediator of lethal systemic inflammation in sepsis. Since glutamine (GLN) was shown to have anti-inflammatory and antioxidant properties, we hypothesized that GLN administration may downregulate an HMGB-1-mediated pathway and thus ameliorate sepsis-induced AKI. Mice were randomly assigned to a normal group (NC), a septic saline group (SS), or a septic GLN group (SG). Sepsis was induced by cecal ligation and puncture (CLP). The SS group was injected with saline, and the SG group was given 0.75 g GLN/kg body wt once via a tail vein 1 h after CLP. Mice were killed 2, 6, and 24 h after CLP, and blood and kidneys of the animals were harvested for further analysis. The results showed that sepsis resulted in higher mRNA and/or protein expressions of kidney HMGB-1, toll-like receptor (TLR) 4, myeloid differentiation primary-response protein (MyD) 88, and receptor of advanced glycation end products (RAGE) compared with normal mice. Septic mice with GLN administration exhibited decreased HMGB-1, TLR4, RAGE, and phosphorylated NF-κB p65 protein expressions and reduced nitrotyrosine levels in kidney tissues. The histological findings showed that damage to the kidneys was less severe, and survival improved in the SG group. These results indicated that a single dose of GLN administered after the initiation of sepsis plays a prophylactic role in downregulating the expressions of HMGB-1-related mediators and decreasing oxidative stress in the kidneys, which may consequently have ameliorated AKI induced by sepsis.

acute kidney injury; glutamine; NF-κB p65

SEPSIS IS CHARACTERIZED BY a severe inflammatory response to infection, and it remains the leading cause of death in critically ill patients. Sepsis causes multiorgan failure, and acute kidney injury (AKI) is a critical complication of sepsis (8). Moreover, sepsis-induced AKI worsens the survival prognosis during sepsis and is associated with 70–80% mortality in intensive care units (10). Until recently, the pathogenesis of AKI in sepsis remained incompletely understood (2). However, vasodilation-induced glomerular hypoperfusion, dysregulated circulation within the peritubular capillary network, inflammatory reactions by cytokine cascades, and tubular dysfunction induced by oxidative stress were proposed (6).

High-mobility group box (HMGB)-1 is a highly conserved ubiquitous nuclear protein present in almost all eukaryotic cells, where it regulates gene expression and transcription under normal conditions (36). Evidence indicates that high levels of HMGB-1 accumulate in animals and humans with severe sepsis, which mediates systemic inflammation, organ failure, and lethality (37). Unlike other proinflammatory cytokines such as TNF-α or IL-1β, HMGB-1 was implicated as a “late” mediator of lethal systemic inflammation and provides a wider time frame for clinical intervention against the progressive inflammatory cascade of sepsis (17). The direct contribution of HMGB-1 to kidney injury was demonstrated by previous studies. They showed that endogenous HMGB-1 promotes kidney damage during inflammation, and administration of a neutralizing antibody to HMGB-1 affords significant protection (3, 8, 24, 35). HMGB-1 released from damaged tissues activates cellular signals by interacting with the receptor for advanced glycation end products (RAGE) and toll-like receptor (TLR)2 and TLR4. TLRs are a family of pattern recognition receptors that detect motifs of pathogens and host materials released during injury that are important in activating innate immunity. These receptors are involved in a myeloid differentiation primary-response protein (MyD) 88-dependent pathway that ultimately leads to NF-κB activation (16). Previous studies reported that NF-κB regulates the expressions of proinflammatory cytokines and chemokines, which cause kidney damage after ischemia-reperfusion injury and sepsis-induced AKI (12, 16). However, the role of HMGB-1 in sepsis-induced AKI remains largely unknown.

Glutamine (GLN) is the most abundant free amino acid in the circulation. Several studies demonstrated that GLN has immunomodulating properties (9, 33). GLN is required during catabolic processes to manifest optimal tissue responses to catabolism, inflammation, and infection and is considered an essential amino acid during certain disease conditions (19, 32). Recently, a study by Kwon et al. (13) found that GLN inhibits HMGB-1 expression and attenuates acute lung injury during sepsis. GLN was shown to be beneficial mostly in preventing gut mucosal and lung injury. Studies investigating the effects of GLN on sepsis-induced AKI are rare. Oliveira et al. (18) revealed that in a cecal ligation and puncture (CLP) rodent model, a single intravenous dose of GLN attenuated epithelial cell apoptosis in the kidneys. A study performed by our laboratory also found that dietary GLN supplementation decreased kidney IL-6 expression in septic mice (38). Here, we hypothesized that GLN administration downregulates HMGB-1-mediated signaling and thus attenuates AKI in gut-derived sepsis. In this study, mRNA and/or protein expressions of

Address for reprint requests and other correspondence: S.-L. Yeh, School of Nutrition and Health Sciences, Taipei Medical Univ., 250 Wu-Hsing St., Taipei, 110 Taiwan (e-mail: sangling@tmu.edu.tw).
Differentiation factor 88; TLR4, toll-like receptor 4; F, forward primer; R, reverse primer.

Materials and Methods

Animal preparations. Ten-week-old male ICR mice, weighing 25–30 g at the beginning of the experiment, were used in this study. All mice were housed in a temperature- and humidity-controlled room and were allowed free access to a standard chow diet for 1 wk before the study. Care of laboratory animals was in full compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996), and protocols were approved by the Institutional Animal Care and Use Committee at Taipei Medical University.

Experimental procedures. Mice were randomly assigned to a normal group (NC, n = 8), a septic saline group (SS; n = 24), and a septic GLN group (SG; n = 24). Polymicrobial sepsis by CLP was induced in mice in the SS and SG groups. CLP was performed as previously described (25). Briefly, mice were anesthetized with an intraperitoneal injection of ketamine (40 mg/kg) and xylazine (6 mg/kg, Virbac, Carros, France), a 1-cm incision was made in the abdominal wall, and the cecum was extruded. Approximately 25% of the cecum was ligated just below the ileocecal valve with 3-0 silk. The induction in mice in the SS and SG groups. CLP was performed as previously described (25). Briefly, mice were anesthetized with an intraperitoneal injection of ketamine (40 mg/kg) and xylazine (6 mg/kg, Virbac, Carros, France), a 1-cm incision was made in the abdominal wall, and the cecum was extruded. Approximately 25% of the cecum was ligated just below the ileocecal valve with 3-0 silk. The distal cecum was then punctured in two places with a 22-gauge needle to allow a small amount of fecal material to extrude into the peritoneal cavity, after which it was replaced into the abdomen. The abdominal wound was closed in two layers. The animals were given subcutaneous fluid resuscitation (40 ml/kg body wt) during the postoperative period. All of the above manipulations were performed by the same person to ensure consistency. This length of the ligated cecum was chosen because it consistently yielded ~75% mortality in control animals (26). After the operation, the SS group was injected with saline, and the SG group was given 0.75 g GLN/kg body wt once via a tail vein 1 h after CLP. GLN was administered as alanyl-glutamine dipeptide (Dipeptiven concentrated solution for infusion; Fresenius-Vilnius, Lithuania) according to standard protocols. RT was carried out by subsequent incubation for 5 min at 70°C, 5 min at 37°C, 60 min at 42°C, and 10 min at 70°C. cDNA was stored at −80°C until used. Specific mRNA genes were amplified by real-time RT-PCR using the 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA) with SYBR Green I as the detection format. Primers of the housekeeping gene (β-actin), HMGB-1, RAGE, heat shock protein (HSP) 70, Myd88, and TLR4 were purchased from Mission Biotech (Taipei, Taiwan) based on standard protocols. NCBI. Primer sequences used for the quantitative RT-PCR assays are listed in Table 1. Amplification was carried out in a total volume of 25 μl containing 1× Power SYBR Green PCR Master Mix (Applied Biosystems), 400 nM of each primer, and 100 ng of cDNA. The reaction was performed by 1 cycle of 2 min at 50°C and 10 min at

<table>
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<tr>
<th>Gene Name</th>
<th>Accession No.</th>
<th>5'–3' Primer Sequence</th>
<th>Product Size, bp</th>
</tr>
</thead>
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<tr>
<td>β-Actin</td>
<td>NM_007393.2</td>
<td>F: ACCCATGATCAGTGACCCATCC &lt;br&gt; R: TCTCGAGCTGGTGTGGTGA</td>
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<td>NM_007425.2</td>
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<td>Myd88</td>
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HMGB-1, high mobility group box protein-1; RAGE, receptor of advanced glycation end products; HSP70, heat shock protein 70; Myd88, myeloid differentiation factor 88; TLR4, toll-like receptor 4; F, forward primer; R, reverse primer.

Measurements of plasma biochemical parameters. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were determined using commercial kits (Randox Laboratories, Crumlin, Antrim, UK). Plasma creatinine (Cr) and blood urea nitrogen (BUN) were also measured (BioAssay Systems, Hayward, CA). Procedures followed the manufacturer’s instructions.

Analysis of nitrotyrosine in kidney homogenates. Kidney tissues were homogenized (1:3, wt/vol) in ice-cold lysis buffer (50 mM Tris-HCl; pH 7.4) containing a protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). The homogenates were centrifuged at 15,000 rpm for 20 min, and the cell debris was discarded. Supernatants were used for the analysis of nitrotyrosine. Nitrotyrosine concentrations were measured using a commercial ELISA kit (Millipore, Bedford, MA). Nitro-BSA was coated onto wells of microtiter strips, and nitrotyrosines were quantitated using an anti-nitrotyrosine antibody. Competition was accomplished by adding 50 μl of supernatant and 50 μl of the primary antibody to the wells. Each competes with the coated nitrated proteins for antibody binding. The amount of antibody that binds to the coated nitro-BSA is inversely proportional to the amount of nitrotyrosine present in the samples added to the wells of the plate. Procedures followed the manufacturer’s instructions.

Table 1. Primer sequences used in the quantitative RT-PCR assays

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For 30 min, and blots were developed with high-sensitivity chemiluminescence substrate Western Lighting Ultra (PerkinElmer Life Sciences, Waltham, MA) and exposed to X-ray films. The relative intensity was measured to quantify the protein level. All blots were normalized against actin to adjust for the amount of protein loaded.

**Histological examination.** Histological analysis of the kidneys was conducted after organ removal, tissue fixation by immersion in 4% buffered paraformaldehyde, and embedding in paraffin. Five-micrometer paraffin-embedded kidney sections were stained with periodic acid-Schiff reagent (PAS; Sigma), and standard hematoxylin (Sigma) and periodic acid-Schiff reagent (PAS; Sigma) and standard hematoxylin (Sigma). anti-HMGB-1 (Epitomics, Burlingame, CA), and anti-inducible nitric oxide synthase (iNOS; 1:200; Santa Cruz Biotechnology) antibodies overnight, or a mouse anti-actin antibody (1:10,000; Sigma) for 1 h. After the membranes were washed three times (for 10 min each) in TBS-T, a goat anti-rabbit IgG or anti-mouse IgG-horseradish peroxidase conjugate (Millipore) was applied at a 1:2,000 dilution.

**Table 2. Blood urea nitrogen, creatinine, aspartate transaminase, and alanine transaminase in plasma during sepsis**

<table>
<thead>
<tr>
<th></th>
<th>BUN mg/dl</th>
<th>Cr mg/dl</th>
<th>AST U/l</th>
<th>ALT U/l</th>
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<tr>
<td>NC</td>
<td>23.6 ± 4.32</td>
<td>0.25 ± 0.05</td>
<td>43.30 ± 19.07</td>
<td>8.93 ± 4.71</td>
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<td>SS</td>
<td>33.71 ± 2.93† ‡</td>
<td>0.38 ± 0.08†</td>
<td>45.33 ± 10.62</td>
<td>9.27 ± 1.72</td>
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<tr>
<td>SG</td>
<td>33.61 ± 3.66†</td>
<td>0.35 ± 0.08†</td>
<td>39.26 ± 9.93</td>
<td>6.79 ± 1.49</td>
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<tr>
<td>6 h</td>
<td>43.85 ± 6.07†</td>
<td>0.38 ± 0.06†</td>
<td>39.95 ± 7.47</td>
<td>14.11 ± 1.70</td>
</tr>
<tr>
<td>SS</td>
<td>36.61 ± 3.74* ‡</td>
<td>0.27 ± 0.07*</td>
<td>46.34 ± 10.78</td>
<td>8.62 ± 2.62</td>
</tr>
<tr>
<td>SG</td>
<td>37.47 ± 3.82* ‡</td>
<td>0.26 ± 0.04*</td>
<td>51.91 ± 12.96*</td>
<td>21.56 ± 5.83*</td>
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</table>

Values are means ± SD expressed as mg/dl for blood urea nitrogen (BUN) and creatinine (Cr) and U/l for aspartate transaminase (AST) and alanine transaminase (ALT). NC, normal control group; SS, septic saline group; SG: septic glutamine group. *Significantly different from the SS group at the same time point. †Significantly different from the NC group. ‡Significantly different from the same group at different time points. P < 0.05.

**Fig. 1. Survival rates for 48 h of animals after cecal ligation and puncture (CLP). Survival rates in the septic glutamine (SG) group were significantly higher than those in the septic saline (SS) group according to the Kaplan-Meier log-rank test ( * P < 0.05).**

**Fig. 2. Concentrations of nitrotyrosine in kidney homogenates at different times after CLP. Values are means ± SD. Differences among groups were analyzed by ANOVA followed by a Newman-Keuls multiple-comparison test. Different groups with different time schedules were analyzed by 2-way ANOVA using a Bonferroni post hoc test. *Significantly different from the SS group at the same time point. †Significantly different from the normal control (NC) group. ‡Significantly different from the same group at different time point (P < 0.05).**
nuclear staining was also applied to contrast cell nuclei. Histological changes in the cortex and medulla were assessed by quantitative measurements of kidney damage. Tubular damage was defined as tubular epithelial swelling, loss of brush borders, vacuolar degeneration, necrotic tubules, cast formation, and desquamation. To demonstrate HMGB-1 immunoreactivity, Formalin-fixed paraffin-embedded sections (5 μm) were deparaffinized and boiled for 10 min in 10 mM sodium citrate buffer. All tissue sections were preincubated in a blocking solution containing 10% normal goat serum and 0.3% H2O2 in 0.1 M phosphate buffer for 1 h to block endogenous peroxidase activity and nonspecific binding of antibodies. Sections were then incubated with a rabbit monoclonal primary antibody against HMGB-1 (Epitomics), and diluted 1:300 in 0.1 M phosphate buffer overnight at 4°C. Immunostaining was visualized by detection of peroxidase activity. For peroxidase activity detection, tissue sections were incubated in biotinylated goat anti-rabbit IgG (diluted 1:300, Chemicon, Temecula, CA) for 1 h at room temperature. After reacting with the peroxidase-linked Avidin-Biotin complex (Vector, Burlingame, CA) for 1 h at room temperature, a diaminobenzidine solution kit (Vector) was used to detect HMGB-1 immunoreactivity. Hema-toxylin (Sigma) nuclear staining was also applied to contrast the cell nucleus and cytoplasm. The degree of kidney damage and the intensity of the HMGB-1 immunoreactivity were estimated at ×200 magnification using at least 10 microscopic fields/section and three to six independent samples for each group measured by a digital image-analysis system (Image Pro Plus 5.1, Media Cybernetics, Silver Spring, MD). Values are expressed as micrometers squared, and average areas were obtained for each group.

Statistical analysis. Data are expressed as means ± SD. Survival curves were compared using a log-rank test. Differences among groups were analyzed by ANOVA followed by a Newman-Keuls multiple-comparison test. Different groups with different time schedules were analyzed by two-way ANOVA using a Bonferroni post hoc test. A P value of <0.05 was considered statistically significant. All analyses were conducted using GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

RESULTS

Body weights. There were no differences in the initial body weights among the three groups (data not shown).

Survival rates. No mortality occurred in the NC group. In the SG group, 56% had survived at 48 h post-CLP, which was significantly different from the septic saline (SS) group (P < 0.05).†Significantly different from the NC group (P < 0.05). Differences between groups were analyzed by ANOVA with a Newman-Keuls multiple-comparison test.

Fig. 3. Western blot analysis of high-mobility group box protein (HMGB)-1, receptor of advanced glycation end products (RAGE), toll-like receptor (TLR)4, and inducible nitric oxide synthase (iNOS) protein in kidneys 24 h after CLP. Equal loading of proteins is illustrated by β-actin bands. Relative densities are presented as means ± SD by densitometric analysis of 6 animals/group. *Significantly different from the septic saline (SS) group (P < 0.05). †Significantly different from the NC group (P < 0.05). Differences between groups were analyzed by ANOVA with a Newman-Keuls multiple-comparison test.

Fig. 4. Western blot analysis of phospho-NF-κB p65 (serine 536) and IκBα proteins in the kidneys 24 h after CLP. Equal loading of proteins is illustrated by β-actin bands. Relative densities are presented as means ± SD by densitometric analysis of 6 animals/group. *Significantly different from the SS group (P < 0.05). †Significantly different from the NC group (P < 0.05). Differences between groups were analyzed by ANOVA with a Newman-Keuls multiple-comparison test.
significantly higher than the 22% observed in the SS group (Fig. 1).

**Plasma levels of biochemical parameters.** Plasma BUN and Cr levels were significantly higher in the septic groups at various time points after CLP than in the NC group. Also, plasma AST and ALT activities were higher in the SS group than the NC group. Compared with the septic group without GLN, GLN administration produced lower BUN and Cr at 6 and 24 h, whereas AST and ALT were lower at 24 h after CLP (Table 2).

**Nitrotyrosine levels in the kidneys.** Nitrotyrosine levels at 2 and 6 h after CLP were lower in the SG than the SS group (Fig. 2).

**Protein expressions in the kidneys.** In the SS group, HMGB-1, RAGE, TLR4, and iNOS protein expressions were significantly higher than those in the NC group. The expressions of these proteins were significantly lower in the SG group than the SS group 24 h after CLP (Fig. 3). Compared with the NC group, the SS group showed significantly lower 1xBax and higher phospho-NF-κB p65 expressions. The SG group had higher 1xBax and lower phospho-NF-κB p65 expressions than the SS group 24 h after CLP (Fig. 4).

**Gene expressions in the kidneys.** The SS group had higher HMGB-1, RAGE, MyD88, and TLR4 mRNA expressions than those of the NC group. The expressions of these genes were significantly lower in the SG group than the SS group. The SG group had higher HSP70 mRNA expression than the NC and SS groups (Fig. 5).

**Morphological aspects of the kidneys.** PAS techniques were used to demonstrate polysaccharides, neutral mucosubstances, microvilli, and basement membranes primarily in tissue. Higher PAS-positive areas of tubular microvilli, basal lamina indicated more intact structures in renal tissues. Purple PAS-stained areas with blue hematoxylin-stained nuclei were seen in sepsis-induced kidney tissues. Morphological changes and quantified results of PAS-stained areas among groups are shown in Fig. 6. Histological findings in normal kidneys showed renal tubules with no inflammatory changes. Slightly dilated renal tubules and swollen tubular cells were observed in the SS group, and thickened basement membranes of renal tubules were found in the SG group. PAS-stained findings showed that NC and SG groups exhibited greater PAS-positive intensity than the SS group, especially on the apical border of the tubular epithelium. The quantitative analysis of the PAS-stained area demonstrated that the SS group (28,146 ± 466 μm²/field) had significantly smaller stained areas compared with the SG (30,768 ± 288 μm²/field) and NC groups (30,586 ± 825 μm²/field) (P < 0.05). Immunocytochemical findings of brown-stained HMGB-1-immunoreactive cells and hematoxylin-stained nuclei were also seen in kidney tissues to check the location changes in HMGB-1. The HMGB1-positive cells were mostly observed in the renal tubules. In the NC and SG groups, HMGB-1 was noted predominantly in the nucleus of renal parenchyma cells, especially renal tubular epithelial cells. However, in the SS group, cytoplasmic HMGB-1 increased and high levels of extracellular HMGB-1 were also detected. The immunoreactive intensities of HMGB-1 were highest in the SS group (Fig. 7A). Quantification results for HMGB-1-immunoreactive areas among groups are shown in Fig. 7B. The immunoreactive areas of the SS group were significantly larger than those of the NC and SG groups.

**Fig. 5.** mRNA expressions of HMGB-1 (A), RAGE (B), HSP70 (C), MyD88 (D), and TLR4 (E) in kidneys 24 h after CLP measured by real-time RT-PCR. All data are representative of duplicate measurements and are presented as means ± SD (n = 4–6). Differences among groups were analyzed by ANOVA followed by a Newman-Keuls multiple-comparison test.

*Significantly different from the SS group.
†Significantly different from the NC group (P < 0.05).
DISCUSSION

In this study, we used CLP as a sepsis model. CLP is currently the most widely used animal model of sepsis, because CLP more closely mimics the clinical conditions of meningo-coccal sepsis or postsurgical peritonitis in human patients (6). Because the usage of antibiotics may prolong the development of AKI (5), antibiotics were not applied to the animals after CLP. This model was used in some studies investigating the inflammatory reaction in CLP-induced AKI (10, 18); however, this model may not meet clinical standards in the treatment of sepsis. We did not include a sham operation group but instead used chow-fed healthy mice as the control. Since CLP is a well-established gut-derived sepsis model, there seemed no need to confirm that sepsis was successfully induced. Our study design can be used to assess whether GLN supplementation restored kidney injury comparable to the normal control mice. We chose to analyze HMGB-1-associated mediators 24 h post-CLP because a previous study showed that HMGB-1 expression increased at 18–24 h, and NF-κB slowly increased in the kidneys over 24 h. Also, plasma Cr and BUN levels were elevated at this time point, indicating that AKI can be observed at 24 h in a CLP mice model (14).

Results of this study showed that sepsis resulted in higher mRNA and/or protein expressions of HMGB-1, TLR4, MyD88, HSP70, and RAGE in the kidneys compared with those of normal mice. These findings were consistent with a study performed by Susa et al. (28), who also found that RAGE and HMGB-1 in the rat diaphragm were elevated after CLP. In pathological conditions, HMGB-1 is actively secreted by activated macrophages or released from necrotic and damaged cells. Extracellularly released HMGB-1 can bind to receptors like RAGE, TLR2, and TLR4, resulting in signal transduction that elicits inflammatory responses. TLR4 was implicated as the critical receptor mediating the inflammatory activity of HMGB-1 (36). RAGE is a multiligand receptor of the Ig superfamily of cell-surface molecules. A previous study revealed that neutralization of RAGE and HMGB-1 attenuates diaphragm dysfunction in intra-abdominal sepsis (28). In addition, one experimental study indicated that HMGB-1 induces cytokine production and neutrophil recruitment in vivo by a mechanism that depends, at least in part, on TLR4 and RAGE (31). MyD88 is a central adaptor protein for the majority of TLRs and RAGE, acting as a link between the receptors and downstream kinases (1). In a MyD88-dependent manner, HMGB-1-mediated signaling leads to NF-κB activation (16). In resting cells, NF-κB is sequestered in the cytoplasm in an inactive state bound to its inhibitor protein, IκB. Upon stimulation, Iκ-Bα is phosphorylated and degraded, resulting in the release and translocation of NF-κB to nuclei (15). Phosphorylation of the p65 subunit of NF-κB may contribute to complete p65 activation and subsequent DNA binding which activates the transcription of target genes, such as cytokines, iNOS, RAGE, etc. (23). In the present study, we found that the renal phospho-NF-κB p65 protein level increased, whereas...
IkBα protein decreased in sepsis indicating that HMGB1-related mediators were activated in CLP-induced AKI. A study by Dear et al. (4) reported that AKI induced by polymicrobial sepsis is dependent on MyD88, but not TLR4. However, a study by El-Achkar et al. found that TLR4 expression markedly increased in all proximal and distal tubules, glomeruli, and the renal vasculature in CLP rats (7). Our study design cannot differentiate the influences of respective mediators on AKI. The higher TLR4 accompanied by other mediator expressions observed in this study indicated that at least an inflammatory response in kidney tissues had been activated. We speculated that iNOS may partly be involved in the occurrence of AKI, because a previous report revealed that higher iNOS expression was responsible for alterations in regional and systemic hemodynamics and impairment of organ function in sepsis (10).

Several studies were undertaken with a view to preventing the progression of multiorgan dysfunction during sepsis by inhibiting HMGB-1, RAGE (28), ethyl pyruvate, or its analog (14, 30). To determine the effects of GLN on the HMGB-1-mediated signaling cascade, we used a single 0.75 g/kg dose of intravenous GLN. This dose was found to result in a plasma GLN level of 3–7 mM/l within 15 min (34), attenuate acute lung injury, and improve survival in a sepsis rodent model (25). In this study, we found that GLN administration suppressed HMGB-1, TLR4, MyD88, and RAGE expressions. Also, the phospho-NF-κB p65 protein level decreased in kidney tissues. A previous study showed that inhibition of NF-κB ameliorates sepsis-induced AKI (10). These results indicate that GLN administration downregulated the expression of HMGB-1-mediated proteins in kidney tissues, consequently leading to a reduction in the inflammatory response and higher survival rates in sepsis. Our results are consistent with the report by Kessel et al. (11), who also found that treatment with GLN downregulates intestinal TLR4 and MyD88 expressions caused by LPS endotoxemia. Since inhibitors specifically against HMGB-1-related mediators were not used in this study, we cannot be sure that HMGB-1 is the direct target of GLN, and the possibility that GLN has effects on pathways other than HMGB-1 cannot be excluded.

HSPs are a family of proteins involved in the most basic mechanisms of cellular protection. Clinical data indicated that increased levels of serum HSP70 correlate with improved survival in patients following severe trauma (21). HSP70 overexpression has been shown to inhibit HMGB-1 release from macrophages in response to stimuli (29). A recent study reported that GLN supplementation enhances the HSP70 response and concomitantly inhibits HMGB-1 expression in septic animals (27). Our results support the description that higher HSP70 and lower HMGB-1 expressions were observed in the septic group with GLN administration. GLN is a precursor for the synthesis of glutathione (GSH). GLN was found to be rate limiting for GSH synthesis, and the availability of GLN is critical for generating GSH stores (22). Yu et al. (39) found that GLN-supplemented nutrition preserved GSH stores after treatment with an antineoplastic agent. In this study, we found that kidney nitrotyrosine levels in the early phase of

Fig. 7. A: immunohistochemical staining of HMGB-1 in kidney tissue 24 h after CLP. The NC and SG groups show intense HMGB-1 expression predominantly in the nucleus of renal parenchyma cells of the tubular epithelium. In the SS group, cytoplasmic HMGB-1 was significantly increased. B: quantitative analysis shows that the SS group had significantly greater HMGB-1-stained areas than the NC and SG groups. *Significantly different from the SS group. †Significantly different from the NC group (P < 0.05).
GLN also improved survival. These benefits of HMGB-1, TLR4, MyD88, RAGE, and NF-κB p65 in kidney tissues. GLN also improved survival. These benefits may be associated with an enhancement of kidney HSP70 levels and a decrease in nitric oxide oxidation during sepsis. The results of the present study provide basic information that GLN can be considered a potential therapeutic agent for preventing AKI induced by sepsis.

**REFERENCES**


