—Original—

Dexmedetomidine Might Exacerbate Acute Kidney Injury, While Midazolam Might Have a Postconditioning Effect: A Rat Model of Lipopolysaccharide-Induced Acute Kidney Injury

Akiko Hata, Makiko Yamamoto, Masae Iwasaki, Tomonori Morita, Masashi Ishikawa and Atsuhiro Sakamoto

Department of Anesthesiology and Pain Medicine, Graduate School of Medicine, Nippon Medical School, Tokyo, Japan

Background: The preconditioning effects of dexmedetomidine and propofol on septic acute kidney injury (AKI) have been reported, but the postconditioning effects remain unknown. This study investigated the postconditioning effects of dexmedetomidine, midazolam, and propofol on septic AKI.

Methods: Forty-eight male Wistar rats were intraperitoneally administered lipopolysaccharide (LPS; 8.3 mg kg^{-1}) or normal saline. Twenty-four hours later, rats were allocated to specific anesthetic groups (n= 6 each) and exposed for 6 h, as follows: C, control (no anesthetic); D, dexmedetomidine (5 μg kg^{-1} h^{-1}); M, midazolam (0.6 mg kg^{-1} h^{-1}); or P, propofol (10 mg kg^{-1} h^{-1}). Serum creatinine (Cr) and cystatin C (CysC) were measured at the end of anesthesia. Western blot and immunofluorescent analyses of kidney samples were performed.

Results: Among LPS-treated groups, D group showed worsened renal dysfunction (L-C vs L-D: Cr, P= 0.002, effect size (η^2)=0.83; CysC, P=0.004, η^2=0.71), whereas M group showed improved renal function (L-C vs L-M: Cr, P=0.009, η^2=0.55). In immunofluorescent analysis of renal tubules, D group showed increased expression of nuclear factor κB (NFκB) (L-C vs L-D: NFκB, P=0.002, η^2=0.75; phospho-NFκB, P= 0.018, η^2=0.66) and inhibitor of κ light polypeptide gene enhancer in B-cell kinase β (IKKβ) (L-C vs L-D: IKKβ, P=0.002, η^2=0.59; phospho-IKKα/β, P=0.004, η^2=0.59), whereas M group showed decreased NFκB expression (L-C vs L-M: NFκB, P=0.003, η^2=0.55; phospho-NFκB, P=0.013, η^2=0.46).

Conclusions: Dexmedetomidine administration might worsen septic AKI, while midazolam might preserve kidney function via the NFκB pathway. (J Nippon Med Sch 2023; 90: 387–397)

Key words: acute kidney injury, dexmedetomidine, lipopolysaccharide, midazolam, sepsis

Introduction

Sepsis is a leading cause of death worldwide, with a 20% mortality rate among hospitalized adults[1]. Sepsis accounts for 40-50% of all cases of acute kidney injury (AKI), increasing the mortality rate to 60%[2,3]. Septic AKI can cause shock status, systemic inflammatory response syndrome, and local organ injury such as inflammation and damage to renal tubules. Important elements in AKI pathology include the induction of both inflammation and an excessive immune response, microcirculatory disturbances, and cell death including necrosis and apoptosis/mitochondrial damage, which can lead to major organ damage (including the kidneys) through the toll-like receptor 4/nuclear factor κB (NFκB) activation pathway. NFκB is widely known as a regulator in the acute phase of inflammatory reactions such as sepsis[4].

Dexmedetomidine, midazolam, and propofol are widely used intravenous anesthetics in intensive care and perioperative settings, but the appropriate sedation and dosage must be considered with care, as some agents and dosages can exacerbate severe hypotension and microcirculatory disturbances caused by sepsis. In the lipopolysaccharide (LPS)-induced model of sepsis, the common intravenous anesthetic agents dexmede-

Correspondence to Akiko Hata, Department of Anesthesiology and Pain Medicine, Graduate School of Medicine, Nippon Medical School, 1–1–5 Sendagi, Bunkyo-ku, Tokyo 113–8602, Japan
E-mail: s11-113wa@nms.ac.jp
https://doi.org/10.1272/jnms.JNMS.2023_90-406
Journal Website (https://www.nms.ac.jp/sh/jnms/)

tomidine[5–12] and propofol[13] inhibit activation of the NFκB pathway and decrease oxidative stress reactions or production of tumor necrosis factor-α (TNFα). Those previous reports involved animal research in which the anesthetic agents were administered before the onset of sepsis, thus representing preconditioning effects. The postconditioning effects of anesthetics after the establishment of septic AKI are still unclear, even though this clinical situation is much more common.

The present study aimed to investigate and compare the postconditioning effects of dexmedetomidine, midazolam, and propofol in a rat model of septic AKI, focusing on the NFκB activation pathway.

Materials and Methods

Ethics Statement

All protocols for animal experiments were approved by the Animal Research Committee at Nippon Medical School (approval no. 2020-028; approval date: 01 April 2020). All experimental protocols were performed in accordance with the ARRIVE guidelines[14].

Animal Welfare and LPS Administration

A total of 57 male Kwl:Wistar rats (mean±standard deviation body weight, 295±9.7 g; age, 9 weeks; Tokyo Laboratory Animals Science Co., Tokyo, Japan) were kept in cages at 26°C and 40-70% humidity under a 12-h:12-h light:dark cycle in a specific pathogen-free facility, with ad libitum access to food and water. Rats were randomly assigned to eight groups: control (C); dexmedetomidine (D); midazolam (M); propofol (P); LPS-treated control (L-C); LPS-treated dexmedetomidine (L-D); LPS-treated midazolam (L-M); or LPS-treated propofol (L-P). LPS-treated groups received intraperitoneal injection of LPS O 55 (8.3 mg kg^{-1}; FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan) dissolved in 1 mL of normal saline. Groups that did not receive LPS were administered 1 mL of normal saline intraperitoneally. After injection, rats had ad libitum access to food and water for 24 h, during which time the AKI should have become established[15]. Examiners were not blinded to group allocation during subsequent experiments. Nine rats that died within 24 h after LPS administration were excluded. The remaining 48 rats were included in this study (n=6 per group).

Anesthesia, Analgesia, and Euthanasia Protocol

An intravenous line was secured from the tail vein in each rat, and maintenance fluid (normal saline, 1 mL h^{-1}) was administered with each anesthetic for 6 h, with or without prior LPS injection: C, no anesthesia; D, dexmedetomidine at 5 μg kg^{-1} h^{-1}; M, midazolam at 0.6 mg kg^{-1} h^{-1}; and P, propofol at 10 mg kg^{-1} h^{-1} (n=6). The dose of each anesthetic was determined according to results from previous reports using rats[16–19].

Sedation depth was assessed using the Rat Grimace Scale without stimulation every hour[20]. At the end of anesthesia, noninvasive blood pressure and heart rate were measured at the tail. Blood and kidney samples were collected under anesthesia with 5% sevoflurane, after which the rats were killed by exsanguination.

Measurement of Serum Creatinine (Cr) and Cystatin C (CysC)

Serum levels of Cr were measured using the enzymatic method, and serum levels of CysC were measured by enzyme-linked immunosorbent assay in accordance with the protocol of the manufacturer (Oriental Yeast Co., Tokyo, Japan). Detailed information on these methods is provided in the **Supplementary material** (https://doi.org/10.1272/jnms.JNMS.2023_90-406).

Western Blot Analysis

Protein was extracted from kidney samples using cell lysis buffer (Cell Signaling Technology, Danvers, MA, USA) and quantified by the Bradford method using SoftMax Pro/SpectraMax (Molecular Devices, San Jose, CA, USA). Forty micrograms of protein sample was loaded onto a 10% Mini-PROTEAN TGX precast gel (Bio-Rad, Hercules, CA, USA) for electrophoresis. After electrophoresis, protein samples were transferred to a polyvinylidene difluoride membrane using the TransBlot Turbo Transcription System (Bio-Rad). Membranes were blocked with 5% enhanced chemiluminescence (ECL) prime blocking reagent (GE Healthcare, Chicago, IL, USA) in Tris-buffered saline containing Tween (Bio-Rad) for 30 min at room temperature, and incubated overnight at 4°C with each primary antibody, namely, anti-phospho-NFκB (pNFκB); anti-NFκB; anti-phospho inhibitor of κ light polypeptide gene enhancer in B-cells kinase α/β (pIKKα/β), anti-IKKβ; anti-TNF receptor-associated factor 6 (TRAF6) primary antibody (1:1,000; Cell Signaling Technology). This was followed by incubation with horseradish peroxidase-conjugated secondary antibody (1:2,000; Cell Signaling Technology) for 1 h at room temperature. GAPDH was selected as an endogenous control (Cell Signaling Technology). Detailed antibody information is listed in the **Supplementary material**. Protein bands were visualized using ECL prime Western Blotting detection reagent (GE Healthcare) and ChemiDoc Touch (Bio-Rad). Optical density was normalized relative to the GAPDH used as a loading standard, and the target protein expression/GAPDH ratio was calculated relative to

the average ratio of the control group of all membranes. The intensity of protein expression was assessed using ImageJ version 1.46 software (National Institutes of Health [NIH], Bethesda, MD, USA). Quantification of image data was performed in a blinded manner.

Immunofluorescent Analysis

Kidney samples were stored in 4% paraformaldehyde, which was then replaced with 40% sucrose solution. Samples were coated with optical cutting temperature compound (Sakura Finetek Japan Co., Tokyo, Japan) and cryo-sectioned at a thickness of 6 μm (Cryostat; Sakura Finetek Japan Co.). Slices were blocked using 5% donkey serum (Sigma-Aldrich, St. Louis, MO, USA) and incubated with each primary antibody (anti-NFκB, anti-pNFκB, anti-pIKKα/β (1:500; Cell Signaling Technology) or anti-IKKβ (Novus Biologicals, Centennial, CO, USA)) overnight at room temperature. Incubation with each secondary antibody (Alexa 488, 1:500; Abcam, Cambridge, UK and Alexa 568, 1:500; Invitrogen, Waltham, MA, USA) was then performed for 1 h at room temperature. Multiple immunostaining was performed to observe the expression of different antigens in the same sample[21]. Nuclear staining was performed using Vectashield mounting medium containing 4 ′, 6-diamidino-2-phenylindole (DAPI) (Merck-Millipore, Darmstadt, Germany). Cells were observed using a BX53/DP74 wide-field fluorescence microscope (Olympus, Tokyo, Japan). A fixed exposure time of 300 msec was used for red pNFκB and red pIKKα/β, 300 msec for green NFκB, and 400 msec for green IKKβ. The black balance was adjusted to compensate for the background. Images were analyzed and quantified using ImageJ version 1.46 software (NIH) and were processed with 50 pixels for blue fluorescent and 450 pixels for red and green fluorescent. The processed images were cropped with each glomerulus and tubule as the region of interest, and target intensity per cell was calculated by dividing total intensity by the number of nucleated cells to obtain target intensity per cell. Three regions were obtained for each sample, and the average value was calculated. Quantification of image data was performed in a blinded manner. Detailed information on these processes is provided in the **Supplementary material**.

Statistical Analysis

To show a 30% change with 80% power at a 5% significance level, a group size of n=6 was needed. Numerical data are shown as mean and standard deviation, and on dot plots. One-way analysis of variance with post-hoc Tukey-Kramer testing was applied for comparisons. A P value of <0.05 was considered to indicate statistical significance. Data were analyzed using GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA).

Results

Anesthetic and LPS Administration Caused No Significant Changes in Vital Signs

All rats survived the anesthesia protocol (n=48). No significant differences in body weight were found between groups. Control groups showed no difference in the degree of sedation with or without LPS administration (C vs L-C: 2.55±0.89 vs 3.56±0.83, P=0.262) (**Fig. 1A**). On the other hand, as compared with the C group, a significant difference in sedation depth was identified in all anesthesia groups with or without LPS administration (C vs D vs M vs P vs L-D vs L-M vs L-P: 2.55±0.89 vs 3.95±0.49 vs 4.00±0.76 vs 4.59±0.43 vs 3.90±0.74 vs 4.03±0.56 vs 4.61±0.41, P=0.035 (C vs D), P=0.026 (C vs M), P<0.001 (C vs P), P=0.046 (C vs L-D), P=0.021 (C vs L-M), P< 0.001 (C vs L-P)) (**Fig. 1A**). All rats in anesthesia groups were kept under moderate sedation (Rat Grimace Scale, 3-5) and showed no significant differences (**Fig. 1A**). Vital signs did not differ between anesthetics with or without LPS administration, as compared with the C group (**Fig. 1B〜1E**).

Dexmedetomidine Administration Exacerbated Septic AKI while Midazolam Had Renoprotective Effects

Renal parameter analysis showed that LPS administration itself exacerbated renal dysfunction (serum Cr: C vs L-C, 0.26±0.02 mg dL^{-1} vs 0.32±0.02 mg dL^{-1}, P=0.005; serum CysC: 1.58±0.20 μg mL^{-1} vs 1.97±0.22 μg mL^{-1}, P= 0.007). Among the combinations of anesthetic agents and LPS administration, dexmedetomidine administration worsened renal dysfunction (serum Cr: L-C vs L-D, 0.32± 0.02 mg dL^{-1} vs 0.39±0.03 mg dL^{-1}, P=0.002; serum CysC: 1.97±0.22 μg mL^{-1} vs 2.55±0.22 μg mL^{-1}, P=0.004) whereas no significant difference was detected with propofol administration (serum Cr: L-C vs L-P, 0.30±0.02 mg dL^{-1} vs 0.30±0.02 mg dL^{-1}, P=0.887; serum CysC: 2.08± 0.18 μg mL^{-1} vs 1.98±0.18 μg mL^{-1}, P=0.842). Midazolam administration alleviated renal dysfunction in terms of serum Cr but not in terms of CysC (serum Cr: L-C vs L-M, 0.31±0.03 mg dL^{-1} vs 0.26±0.03 mg dL^{-1}, P=0.009; CysC: L-C vs L-M: 1.98±0.24 μg mL^{-1} vs 1.90±0.13 μg mL^{-1}, P=0.893) (**Fig. 2**).

Western Blot Analysis Showed No Significant Changes in the NFκB Pathway

Western blot analysis showed no significant differences among anesthetics with or without LPS administration

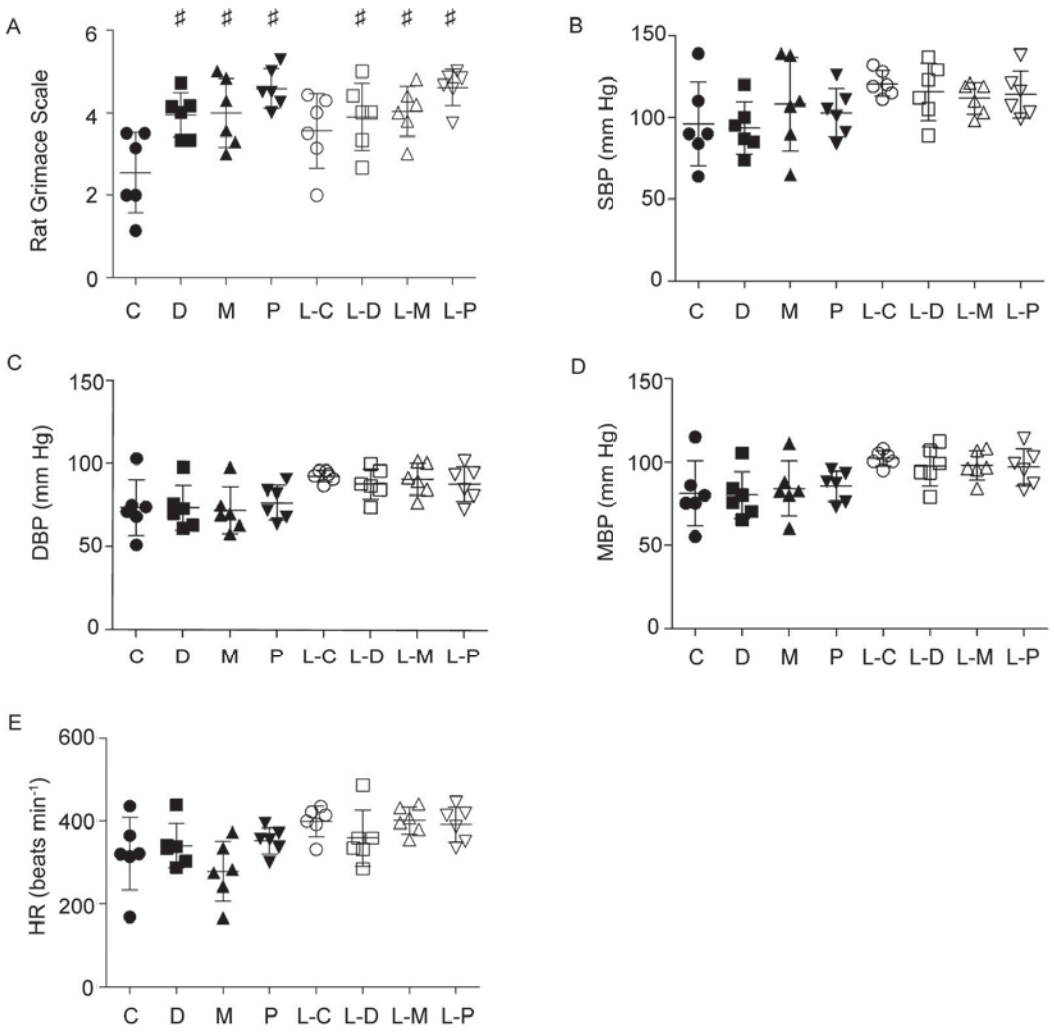

Fig. 1 Sedation depth and vital signs during anesthesia for 6 h with/without LPS administration. **A)** Mean Rat Grimace Scale score assessed every hour during anesthesia. **B)** Mean systolic blood pressure measured from the tail vein at the end of anesthesia (mm Hg). **C)** Mean diastolic blood pressure at the end of anesthesia (mm Hg). **D)** Mean blood pressure at the end of anesthesia (mm Hg). **E)** Mean heart rate measured from the tail vein at the end of anesthesia (beats min^{-1}). C, control; D, dexmedetomidine; M, midazolam; P, propofol; L, lipopolysaccharide (LPS) administration; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate. n=6 per group, one-way analysis of variance with post-hoc Tukey-Kramer test, $P<0.05$, # compared with the C group.

for pNFκB, NFκB, pIKKα/β, IKKβ, or TRAF6 (**Fig. 3**). Since these results may not reflect localized protein expression involved in pathogenesis, we used immunostaining of renal tissue to compare differences in expression.

LPS Administration Itself Stimulated NFκB Activation in Renal Tubules and Glomeruli

To clarify micropathological changes in septic AKI, immunofluorescent analysis of kidney samples was performed (**Fig. 4, 5**; **Supplementary Fig. 1**: https://doi.org/10.1272/jnms.JNMS.2023_90-406). LPS administration increased NFκB activation in renal tubules (C vs L-C: pNFκB, 1.00±0.24 vs 1.64±0.32, P=0.013; NFκB, 1.00 ±0.34 vs 1.84±0.33, P=0.018) (**Fig. 4B, 4C**) without any significant change in IKKβ activation (C vs L-C: pIKKα/β, 1.00 ±0.29 vs 1.24±0.37, P=0.837; IKKβ: 1.00±0.40 vs 1.32±0.48, P=0.633) (**Fig. 4E, 4F**). LPS administration also promoted NFκB activation in renal glomeruli (C vs L-C: pNFκB, 1.00±0.54 vs 1.70±0.33, P=0.042; NFκB, 1.00±0.40 vs 2.45± 0.65, P=0.016) (**Fig. 5B, 5C**) without any significant change in IKKβ activation in renal glomeruli (C vs L-C: pIKKα/β, 1.00±0.52 vs 0.78±0.39, P=0.877; IKKβ, 1.00± 0.98 vs 1.23±0.50, P=0.954) (**Fig. 5E, 5F**).

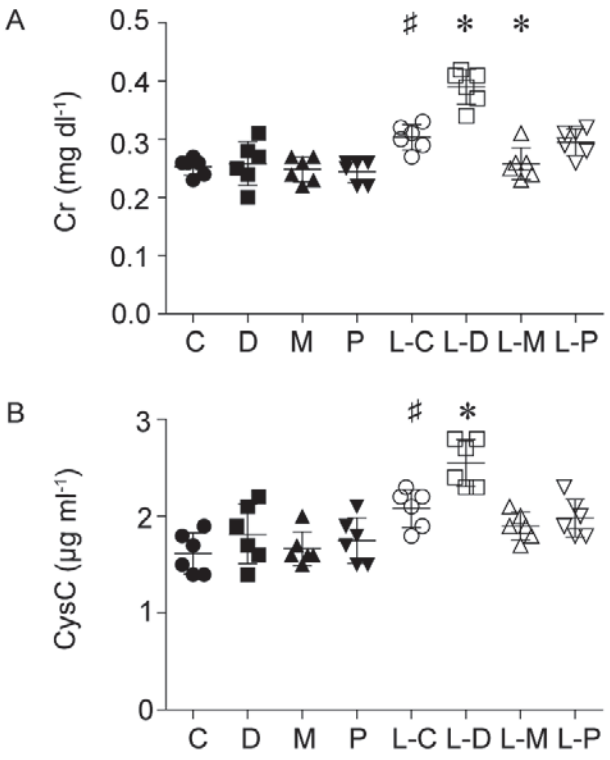

Fig. 2 Renal parameter changes after anesthesia for 6 h with/without LPS administration. **A)** Values of serum creatinine measured by enzyme method (mg dL^{-1}). **B)** Values of serum cystatin C measured by enzyme-linked immunosorbent assay (μg dL^{-1}). C, control; D, dexmedetomidine; M, midazolam; P, propofol; L, lipopolysaccharide (LPS) administration; Cr, serum creatinine; CysC, serum cystatin C. n=6 per group, one-way analysis of variance with post-hoc Tukey-Kramer test, $P<0.05$, # compared with the C group, * compared with the L-C group.

Dexmedetomidine Administration Stimulated, and Midazolam Suppressed, NFκB and IKKβ Activation in Renal Tubules of Septic AKI Rats

In renal tubules, significant differences between D and L-D groups were identified in the expressions of pNFκB, NFκB, and IKKβ, but no such differences were seen between the M and L-M groups or between the P and L-P groups.

Among LPS-treated groups, the L-D group showed the greatest increase in expressions of pNFκB and NFκB in tubules, while the L-M group showed lower expressions than did the L-C and L-D groups. Expressions of pIKKα/β and IKKβ were higher in the L-D group than in the L-C group. The L-M group showed lower expressions of pIKKα/β and IKKβ than the L-D group, with no significant differences as compared with the L-C group (**Table 1A**, **Fig. 4**).

Only Dexmedetomidine Administration Promoted NFκB Activation in Renal Glomeruli of Septic AKI Rats

In glomeruli, significant differences in protein expression in relation to LPS administration in anesthetic groups were found for pNFκB and NFκB with dexmedetomidine and for NFκB with propofol but not with midazolam.

In a comparison of anesthetic agents, only the L-D group showed greater expression of pNFκB than the L-C group. The L-M group had lower expression of pNFκB and NFκB than the L-D group, with no significant difference as compared with the L-C group. Expressions of IKKβ showed no significant differences among LPS-treated groups. The L-M group showed lower pIKKα/β expression than the L-D group but not the L-C group (**Table 1B**, **Fig. 5**).

Discussion

This study compared the renal effects of intravenous anesthetics administered after the onset of LPS-induced septic AKI, focusing on the NFκB pathway. Dexmedetomidine administration exacerbated AKI via activation of the NFκB pathway, while midazolam administration alleviated AKI by suppressing activation of the NFκB pathway in renal tubules. Propofol administration had no obvious effect on LPS-induced AKI. This study revealed differential renal effects of the above anesthetic agents in vivo under identical conditions in a rat model of septic AKI.

Previous studies reported that administering dexmedetomidine before LPS suppressed NFκB activation or other factors via anti-inflammatory and antioxidant effects in renal tissue, indicating the preconditioning effects on AKI[5–7,9–12]. However, in the present study, dexmedetomidine administration after septic AKI establishment exacerbated AKI and increased pIKKα/β, IKKβ, NFκB, and pNFκB expressions in renal tubules. Some clinical trials comparing outcomes among critically ill patients treated with dexmedetomidine, midazolam, and propofol found that dexmedetomidine treatment could induce adverse events, including bradycardia, severe hypotension, and cardiac arrest, possibly leading to reduced renal blood flow[22]. Subclinical data suggest that dexmedetomidine might reduce renal medullary oxygenation as well as renal blood flow in a dose-dependent manner in critically ill adults in intensive care units and healthy sheep[23]. Tissue hypoxia has been reported to activate IKK and NFκB[24]. In this rat model experiment, dexmede-

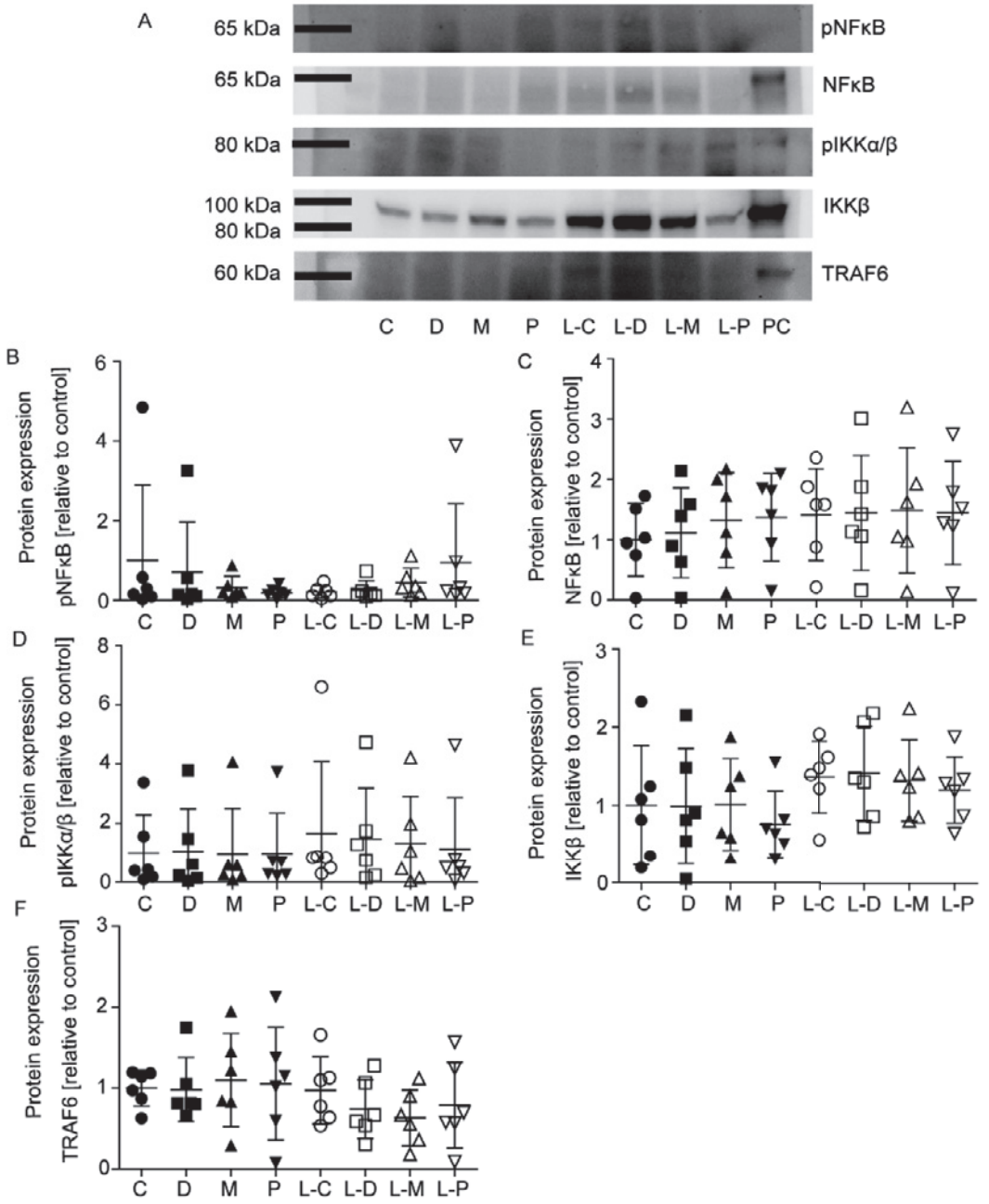

Fig. 3 Western blot analysis of NFκB pathway parameters after anesthesia for 6 h with/without LPS administration. **A)** Representative images of Western blotting assessment of pNFκB, NFκB, pIKKα/β, IKKβ, and TRAF6. **B)** Expression of pNFκB relative to control. **C)** Expression of NFκB relative to control. **D)** Expression of pIKKα/β relative to control. **E)** Expression of IKKβ relative to control. **F)** Expression of TRAF6 relative to control. C, control; D, dexmedetomidine; M, midazolam; P, propofol; L, lipopolysaccharide (LPS) administration; PC, positive control (HeLa whole-cell lysate); NFκB, nuclear factor κB; pNFκB, phospho-nuclear factor κB; IKKβ, inhibitor of κ light polypeptide gene enhancer in B-cell kinase β; pIKKα/β, phospho-inhibitor of κ light polypeptide gene enhancer in B-cell kinase α/β; TRAF6, tumor necrosis factor receptor-associated factor 6. n=6 per group, one-way analysis of variance with post-hoc Tukey-Kramer test, $P<0.05$, # compared with C group, * compared with L-C group.

tomidine administration after onset of LPS-induced sepsis may have similarly caused such vasoactivity as a result of reduced renal blood flow. However, renal oxygenation was not measured, so further studies are needed to clarify these issues.

In terms of midazolam administration before LPS-induced sepsis, several subclinical investigations have shown that midazolam could suppress NFκB pathway

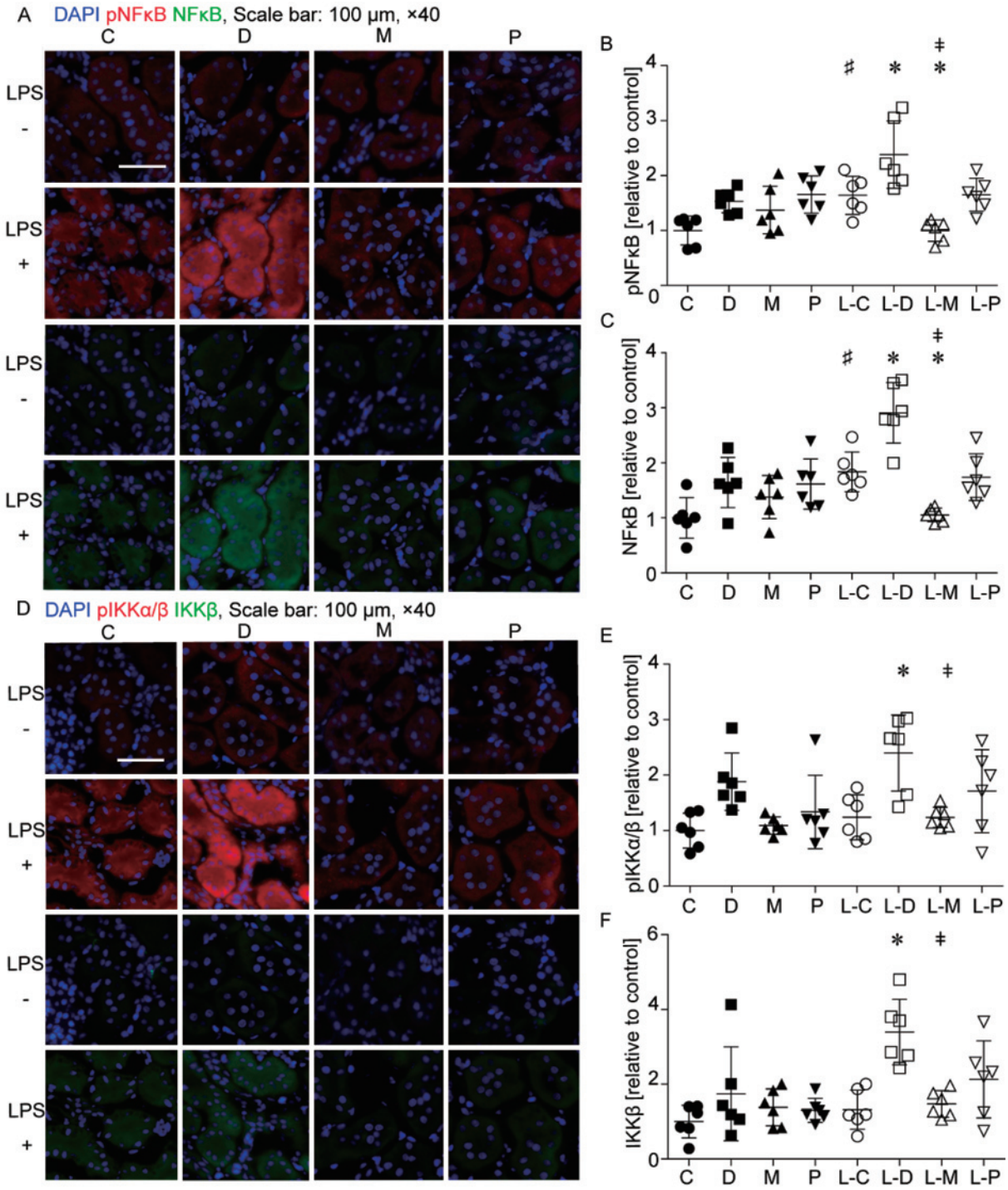

Fig. 4 Immunofluorescent analysis of protein expression in renal tubules after anesthesia for 6 h with/without LPS administration. **A)** Representative immunofluorescent images of renal tubules targeting pNFκB and NFκB. **B)** Immunofluorescent intensity analysis of pNFκB in renal tubules (relative to control). **C)** Immunofluorescent intensity analysis of NFκB in renal tubules (relative to control). **D)** Representative immunofluorescent images of renal tubules targeting pIKKα/β and IKKβ. **E)** Immunofluorescent intensity analysis of pIKKα/β in renal tubules (relative to control). **F)** Immunofluorescent intensity analysis of IKKβ in renal tubules (relative to control). C, control; D, dexmedetomidine; M, midazolam; P, propofol; L, lipopolysaccharide (LPS) administration; NFκB, nuclear factor κB; pNFκB, phospho-nuclear factor κB; IKKβ, inhibitor of κ light polypeptide gene enhancer in B-cell kinase β; pIKKα/β, phospho-inhibitor of κ light polypeptide gene enhancer in B-cell kinase α/β. Red: pNFκB, pIKKα/β; green: NFκB, IKKβ; blue: DAPI. Scale bar=100 μm, ×40 magnification, n=6 per group, one-way analysis of variance with post-hoc Tukey-Kramer test, $P<0.05$, # compared with C group, * compared with L-C group, ‡ compared with L-D group.

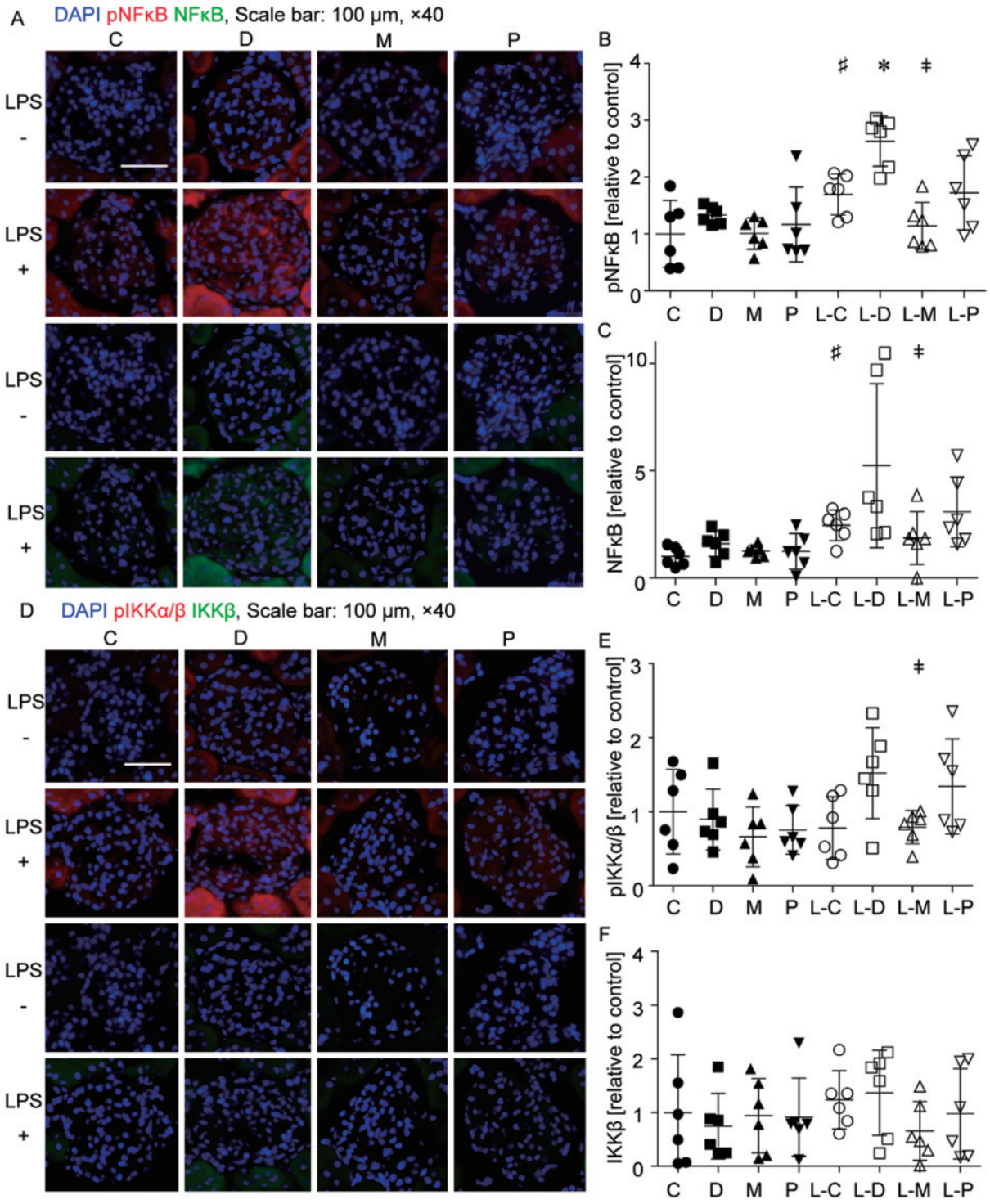

Fig. 5 Immunofluorescent analysis of protein expression in glomeruli after anesthesia for 6 h with/without LPS administration. **A)** Representative immunofluorescent images of renal glomeruli targeting pNFκB and NFκB. **B)** Immunofluorescent intensity analysis of pNFκB in renal glomeruli (relative to control). **C)** Immunofluorescent intensity analysis of NFκB in renal glomeruli (relative to control). **D)** Representative immunofluorescent images of renal glomeruli targeting pIKKα/β and IKKβ. **E)** Immunofluorescent intensity analysis of pIKKα/β in renal glomeruli (relative to control). **F)** Immunofluorescent intensity analysis of IKKβ in renal glomeruli (relative to control). C, control; D, dexmedetomidine; M, midazolam; P, propofol; L, lipopolysaccharide (LPS) administration; NFκB, nuclear factor κB; pNFκB, phospho-nuclear factor κB; IKKβ, inhibitor of κ light polypeptide gene enhancer in B-cell kinase β; pIKKα/β, phospho-inhibitor of κ light polypeptide gene enhancer in B-cell kinase α/β. Red: pNFκB, pIKKα/β; green: NFκB, IKKβ; blue: DAPI. Scale bar=100 μm, ×40 magnification, n=6 per group, one-way analysis of variance with post-hoc Tukey-Kramer test, $P<0.05$, * compared with L-C group, ‡ compared with L-D group.

Table 1 Protein expression results from immunofluorescent analysis

A. Tubules	D vs L-D	M vs L-M	P vs L-P
pNFκB	1.53 (0.19) vs 2.38 (0.56), *P*=0.006*	1.37 (0.39) vs 1.00 (0.18), *P*=0.220	1.65 (0.31) vs 1.65 (0.27), *P*=1.000
NFκB	1.64 (0.42) vs 2.91 (0.50), *P*<0.001*	1.38 (0.36) vs 1.05 (0.11), *P*=0.338	1.61 (0.42) vs 1.74 (0.39), *P*=0.948
pIKKα/β	1.88 (0.47) vs 2.40 (0.63), *P*=0.091	1.09 (0.14) vs 1.24 (0.17), *P*=0.806	1.34 (0.60) vs 1.71 (0.68), *P*=0.664
IKKβ	1.74 (1.15) vs 3.39 (0.80), *P*=0.013*	1.38 (0.45) vs 1.47 (0.33), *P*=0.986	1.30 (0.29) vs 2.13 (0.94), *P*=0.146
	L-C vs L-D	L-C vs L-M	L-D vs L-M
pNFκB	1.64 (0.32) vs 2.38 (0.56), *P*=0.018*	1.64 (0.32) vs 1.00 (0.18), *P*=0.013*	2.38 (0.56) vs 1.00 (0.18), *P*<0.001*
NFκB	1.84 (0.33) vs 2.91 (0.50), *P*=0.002*	1.84 (0.33) vs 1.05 (0.11), *P*=0.003*	2.91 (0.50) vs 1.05 (0.11), *P*<0.001*
pIKKα/β	1.24 (0.37) vs 2.40 (0.63), *P*=0.004*	1.24 (0.37) vs 1.24 (0.17), *P*=1.000	2.40 (0.63) vs 1.24 (0.17), *P*=0.001*
IKKβ	1.32 (0.48) vs 3.39 (0.80), *P*=0.002*	1.32 (0.48) vs 1.47 (0.33), *P*=0.937	3.39 (0.80) vs 1.47 (0.33), *P*=0.003*
B. Glomeruli	D vs L-D	M vs L-M	P vs L-P
pNFκB	1.33 (0.14) vs 2.63 (0.40), *P*<0.001*	1.01 (0.25) vs 1.14 (0.38), *P*=0.903	1.16 (0.60) vs 1.72 (0.60), *P*=0.362
NFκB	1.60 (0.54) vs 5.24 (3.48), *P*=0.026*	1.26 (0.23) vs 1.87 (1.12), *P*=0.520	1.25 (0.76) vs 3.08 (1.48), *P*=0.023*
pIKKα/β	0.90 (0.38) vs 1.52 (0.56), *P*=0.092	0.66 (0.37) vs 0.79 (0.20), *P*=0.949	0.75 (0.30) vs 1.34 (0.59), *P*=0.218
IKKβ	0.75 (0.56) vs 1.37 (0.72), *P*=0.393	0.94 (0.63) vs 0.66 (0.50), *P*=0.880	0.92 (0.66) vs 0.98 (0.77), *P*=0.999
	L-C vs L-D	L-C vs L-M	L-D vs L-M
pNFκB	1.70 (0.33) vs 2.63 (0.40), *P*=0.005*	1.70 (0.33) vs 1.14 (0.38), *P*=0.143	2.63 (0.40) vs 1.14 (0.38), *P*<0.001*
NFκB	2.45 (0.65) vs 5.24 (3.48), *P*=0.101	2.45 (0.65) vs 1.87 (1.12), *P*=0.549	5.24 (3.49) vs 1.87 (1.12), *P*=0.043*
pIKKα/β	0.78 (0.39) vs 1.52 (0.56), *P*=0.089	0.78 (0.39) vs 0.79 (0.20), *P*=1.000	1.52 (0.56) vs 0.79 (0.20), *P*=0.040*
IKKβ	1.23 (0.50) vs 1.37 (0.72), *P*=0.991	1.23 (0.50) vs 0.66 (0.50), *P*=0.548	1.37 (0.72) vs 0.66 (0.50), *P*=0.280

C, control; D, dexmedetomidine; M, midazolam; P, propofol; L, lipopolysaccharide administration; NFκB, nuclear factor κB; pNFκB, phospho-nuclear factor κB; IKKβ, inhibitor of κ light polypeptide gene enhancer in B-cell kinase β; pIKKα/β, phospho-inhibitor of κ light polypeptide gene enhancer in B-cell kinase α/β. n=6 per group, one-way analysis of variance with post-hoc Tukey-Kramer test, *P<0.05

activation in human macrophages and reduce inflammatory mediators[25,26]. We have confirmed that the L-M group, which received midazolam after LPS administration, showed a significant decrease in IL-6 expression in renal tissue compared to the L-C group (**Supplementary Fig. 2**: https://doi.org/10.1272/jnms.JNMS.2023_90-406). These results suggest that administration of midazolam after LPS-induced sepsis may have reduced the inflammatory response and alleviated septic AKI via suppression of NFκB activation in renal tubules.

NFκB is a protein complex that regulates DNA transcription, cytokine production, and cell survival and is associated with inappropriate immune development, including in sepsis[27,28]. Western blot analysis of randomly sampled partial kidneys from each group revealed that the major proteins in the NFκB pathway showed no significant differences in expression, although IKKβ showed signs of a slight increase in expression with the onset of AKI. Therefore, to more precisely analyze fluctuations in the NFκB pathway, detailed localization analysis using fluorescence immunostaining was performed. Immunofluorescence staining allowed clear assessment of the localization of proteins in the NFκB activation pathway. As shown in **Figure 4** and **Supplementary Figure 1**, we believe that NFκB activation occurs at the site of the proximal tubules. However, since specific staining of tissues was not performed, further investigation of localization is needed. Although further validation is needed to identify the mechanisms of pathogenesis, as mentioned above, this study remains valuable as it is the first to reveal postconditioning effects approximating clinical conditions. In addition, the findings highlight the need for physicians to select appropriate anesthetic agents for patients with septic AKI.

In this study, serum Cr and CysC were chosen as indicators of LPS-induced AKI. Serum Cr, which is included in the Kidney Disease: Improving Global Outcomes diagnostic criteria, is widely used for the diagnosis and evaluation of AKI and its severity[29] but might be overestimated under conditions of a mildly reduced glomerular filtration rate (GFR)[30]. Some clinical trials have shown that serum CysC can detect AKI as much as 24 h earlier than Cr[31–33], but this value may be less reliable if GFR is significantly reduced[33]. As the present study observed early AKI after LPS administration, serum CysC was measured simultaneously with Cr, referring to clinical trials[32,33]. Both Cr and CysC levels were significantly elevated in the L-D group. On the other hand, midazolam

significantly alleviated the increase in Cr, although CysC showed no significant difference from the L-C group, despite mean values tending to be slightly lower. One possibility is that the slight improvement in GFR in the L-M group was not reflected in serum CysC levels for the above reasons.

Several limitations to this study must be kept in mind. First, the anesthetic dose and exposure time were fixed, and the exact equipotent dose of anesthetics in rats remained unclear. Dose- and time-dependent effects should therefore be examined. Second, baseline serum Cr and CysC values could not be obtained. Last, continuous blood pressure monitoring could be helpful to rule out potential hypotension during anesthesia, although all groups were lightly sedated and there were no differences in the degree of sedation during the experiment.

In conclusion, dexmedetomidine administration before LPS-induced sepsis exacerbated septic AKI via IKK and NFκB activation, mainly in renal tubules, while midazolam administration had renoprotective effects.

Acknowledgements: We wish to thank Mr. Tatsuki Anjo (TechnoPro, Tokyo, Japan) for his technical support with histological imaging. We are also grateful to Ms. Miyuki Takatori, Ms. Kiyomi Kikukawa, and Ms. Masumi Saito (Collaborative Research Center Laboratory for Clinical Research, Nippon Medical School, Tokyo, Japan) for their help as scientific advisers.

Funding: This work was supported by a KAKENHI grant to Masashi Ishikawa from the Japanese Ministry of Education, MEXT (grant number: 210K08934), Tokyo, Japan.

Conflict of Interest: The authors declare no competing interests.

References

1. Fleischmann C, Scherag A, Adhikari NK, et al. Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. Am J Respir Crit Care Med. 2016 Feb 1;193(3):259–72.
2. Gomez H, Kellum JA. Sepsis-induced acute kidney injury. Curr Opin Crit Care. 2016 Dec;22(6):546–53.
3. Yasuda H, Kato A, Fujigaki Y, Hishida A; Shizuoka Kidney Disease Study Group. Incidence and clinical outcomes of acute kidney injury requiring renal replacement therapy in Japan. Ther Apher Dial. 2010 Dec;14(6):541–6.
4. Matsuda A, Jacob A, Wu R, et al. Novel therapeutic targets for sepsis: regulation of exaggerated inflammatory responses. J Nippon Med Sch. 2012;79(1):4–18.
5. Chen Y, Luan L, Wang C, et al. Dexmedetomidine protects against lipopolysaccharide-induced early acute kidney injury by inhibiting the iNOS/NO signaling pathway in rats. Nitric Oxide. 2019 Apr 1;85:1–9.
6. Feng X, Guan W, Zhao Y, et al. Dexmedetomidine ameliorates lipopolysaccharide-induced acute kidney injury in rats by inhibiting inflammation and oxidative stress via the GSK-3 beta/Nrf2 signaling pathway. J Cell Physiol. 2019 Aug;234(10):18994–9009.
7. Jin YH, Li ZT, Chen H, Jiang XQ, Zhang YY, Wu F. Effect of dexmedetomidine on kidney injury in sepsis rats through TLR4/MyD88/NF-kappaB/iNOS signaling pathway. Eur Rev Med Pharmacol Sci. 2019 Jun;23(11):5020–5.
8. Kiyonaga N, Moriyama T, Kanmura Y. Effects of dexmedetomidine on lipopolysaccharide-induced acute kidney injury in rats and mitochondrial function in cell culture. Biomed Pharmacother. 2020 May;125:109912. Epub 2020 Jan 31.
9. Tan F, Chen Y, Yuan D, Gong C, Li X, Zhou S. Dexmedetomidine protects against acute kidney injury through downregulating inflammatory reactions in endotoxemia rats. Biomed Rep. 2015 May;3(3):365–70. Epub 2015 Feb 12.
10. Yang T, Feng X, Zhao Y, et al. Dexmedetomidine enhances autophagy via alpha2-AR/AMPK/mTOR pathway to inhibit the activation of NLRP3 inflammasome and subsequently alleviates lipopolysaccharide-induced acute kidney injury. Front Pharmacol. 2020;11:790.
11. Yao Y, Hu X, Feng X, et al. Dexmedetomidine alleviates lipopolysaccharide-induced acute kidney injury by inhibiting the NLRP3 inflammasome activation via regulating the TLR4/NOX4/NF-kappaB pathway. J Cell Biochem. 2019 Oct;120(10):18509–23. Epub 2019 Jun 26.
12. Zhao Y, Feng X, Li B, et al. Dexmedetomidine protects against lipopolysaccharide-induced acute kidney injury by enhancing autophagy through inhibition of the PI3K/AKT/mTOR pathway. Front Pharmacol. 2020;11:128.
13. Cui WY, Tian AY, Bai T. Protective effects of propofol on endotoxemia-induced acute kidney injury in rats. Clin Exp Pharmacol Physiol. 2011 Nov;38(11):747–54.
14. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. PLoS Biol. 2020 Jul;18(7):e3000410.
15. Plotnikov EY, Brezgunova AA, Pevzner IB, et al. Mechanisms of LPS-induced acute kidney injury in neonatal and adult rats. Antioxidants (Basel). 2018 Aug 8;7(8):105.
16. Takemoto Y. Dose effects of propofol on hemodynamic and cytokine responses to endotoxemia in rats. J Anesth. 2005;19(1):40–4.
17. Taniguchi T, Kanakura H, Yamamoto K. Effects of post-treatment with propofol on mortality and cytokine responses to endotoxin-induced shock in rats. Crit Care Med. 2002 Apr;30(4):904–7.
18. Taniguchi T, Yamamoto K, Ohmoto N, Ohta K, Kobayashi T. Effects of propofol on hemodynamic and inflammatory responses to endotoxemia in rats. Crit Care Med. 2000 Apr;28(4):1101–6.
19. Qiao H, Sanders RD, Ma D, Wu X, Maze M. Sedation improves early outcome in severely septic Sprague Dawley rats. Crit Care. 2009;13(4):R136. Epub 2009 Aug 19.
20. Sotocinal SG, Sorge RE, Zaloum A, et al. The rat grimace scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Mol Pain. 2011 Jul 29;7:55.
21. Matsuzaki T, Ozawa H. Multiple immunofluorescence labeling in tissue sections. J Nippon Med Sch. 2009 Dec;76(6):278–9.
22. Shehabi Y, Howe BD, Bellomo R, et al. Early sedation with dexmedetomidine in critically ill patients. N Engl J

Med. 2019 Jun 27;380(26):2506–17. Epub 2019 May 19.
23. Plummer MP, Lankadeva YR, Finnis ME, et al. Urinary and renal oxygenation during dexmedetomidine infusion in critically ill adults with mechanistic insights from an ovine model. J Crit Care. 2021 Aug;64:74–81. Epub 2021 Mar 24.
24. Cummins EP, Berra E, Comerford KM, et al. Prolyl hydroxylase-1 negatively regulates IkappaB kinase-beta, giving insight into hypoxia-induced NFkappaB activity. Proc Natl Acad Sci U S A. 2006 Nov 28;103(48):18154–9. Epub 2006 Nov 17.
25. Joo HK, Oh SC, Cho EJ, et al. Midazolam inhibits tumor necrosis factor-alpha-induced endothelial activation: involvement of the peripheral benzodiazepine receptor. Anesthesiology. 2009 Jan;110(1):106–12.
26. Horiguchi Y, Ohta N, Yamamoto S, Koide M, Fujino Y. Midazolam suppresses the lipopolysaccharide-stimulated immune responses of human macrophages via translocator protein signaling. Int Immunopharmacol. 2019 Jan;66: 373–82. Epub 2018 Dec 5.
27. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. Cell. 2008 Feb 8;132(3):344–62.
28. Mitchell S, Vargas J, Hoffmann A. Signaling via the NFkappaB system. Wiley Interdiscip Rev Syst Biol Med. 2016 May;8(3):227–41. Epub 2016 Mar 16.
29. Khwaja A. KDIGO Clinical practice guidelines for acute kidney injury. Nephron Clin Pract. 2012;120(4):c179–84. Epub 2012 Aug 7.
30. Sandilands EA, Dhaun N, Dear JW, Webb DJ. Measurement of renal function in patients with chronic kidney disease. Br J Clin Pharmacol. 2013 Oct;76(4):504–15.
31. Teo SH, Endre ZH. Biomarkers in acute kidney injury (AKI). Best Pract Res Clin Anaesthesiol. 2017 Sep;31(3): 331–44.
32. Ahlström A, Tallgren M, Peltonen S, Pettilä V. Evolution and predictive power of serum cystatin C in acute renal failure. Clin Nephrol. 2004 Nov;62(5):344–50.
33. Ortuno-Anderiz F, Cabello-Clotet N, Vidart-Simon N, Postigo-Hernandez C, Domingo-Marin S, Sanchez-Garcia M. Cystatin C as an early marker of acute kidney injury in septic shock. Rev Clin Esp (Barc). 2015 Mar;215(2):83–90. English, Spanish. Epub 2014 Nov 13.

(Received, November 28, 2022)
(Accepted, April 17, 2023)