Propofol attenuates renal ischemia-reperfusion injury aggravated by hyperglycemia

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ABSTRACT

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Hyperglycemia exacerbates renal ischemia/reperfusion injury via aggravated inflammatory response and excessive production of reactive oxygen species. This study aimed to investigate the ability of propofol, a known antioxidant, to protect kidneys against ischemia/reperfusion injury in hyperglycemic rats in comparison with normoglycemic rats.

Sixty rats were randomly assigned to four groups: normoglycemia-etomidate, normoglycemia-propofol, hyperglycemia-etomidate, and hyperglycemia-propofol. Anesthesia was provided with propofol or etomidate depending on the group. Also, the rats received 1.2 $g \cdot kg^{-1}$ dextrose or the same volume of normal saline depending on the group. Renal ischemia was induced for 25 min. The rats were sacrificed and samples were collected at 65 min after starting IV anesthetics (sham) and at 15 min and 24 h after reperfusion injury to compare the histological degree of renal tubular damage, and levels of inflammatory markers and enzymes related to reactive oxygen species.

Compared to etomidate, propofol significantly attenuated tubular damage after reperfusion in hyperglycemic rats. Also, tubular damage was greater under hyperglycemia compared to normoglycemia in the etomidate group

whereas it was similar in the propofol group. Propofol preserved superoxide dismutase level and attenuated the increase in levels of myeloperoxidase, interlukin-1β, and tumor necrosis factor-α after reperfusion compared to etomidate especially in hyperglycemic rats. Propofol also attenuated production of inducible nitric oxide synthase and phosphorylation of inhibitor of κB and nuclear factor-κB after reperfusion, which were more prominent under hyperglycemia.

Propofol anesthesia conveyed reno-protection against ischemia/reperfusion injury by preserved antioxidation ability and attenuated inflammatory response, which were more prominent under hyperglycemic condition.

Key Words: hyperglycemia, ischemia/reperfusion injury, propofol, renal protection

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I. INTRODUCTION

Ischemia/reperfusion (I/R)-induced renal injury is a frequent and serious complication affecting patients' outcome and resource utilization following cardiovascular or renal allograft surgeries.[1] Although not clearly elucidated, renal I/R is thought to be associated with multiple mechanisms involving reactive oxygen species (ROS) formation,[2] neutrophil infiltration,[2] proinflammatory cytokine generation,[3] and activation of inducible nitric oxide synthase (iNOS),[2] all of which are closely linked to activated nuclear factor (NF)-κB.[4]

In the surgical theatre, hyperglycemia has long been considered as a major risk factor of poor prognosis. Acute and chronic hyperglycemia are associated with impaired nitric oxide (NO) availability[5] and excessive ROS production during I/R.[6] In conjunction, recent studies have depicted that even transient hyperglycemic episodes during I/R in non-diabetic animals significantly intensifies renal injury through increased ROS and proinflammatory cytokines production, and neutrophil activation and infiltration.[7,8] Accounting for the frequent encounter of hyperglycemia in the surgical theatre and its' potential to aggravate renal I/R injury, studies addressing effective protective strategies seem mandatory.

Propofol is a widely used intravenous (IV) anesthetic with an antioxidant activity.[9] Propofol has been shown to decrease plasma proinflammatory cytokine concentrations, ROS production, neutrophil infiltration, iNOS activity, and NF-κB expression.[10,11] Indeed, propofol's reno-protective effect against I/R injury in

normoglycemic condition was superior to that of volatile anesthetics.[11,12] Propofol also attenuated edema formation and lactate accumulation in cerebral I/R injury even in the presence of hyperglycemia,[13] though it is not known whether propofol would mitigate renal I/R injury aggravated by hyperglycemia. Thus, we hypothesized that the antioxidant property of propofol could exert reno-protective effect against I/R injury, even in hyperglycemic condition.

In this study, we investigated the effects of propofol on renal I/R injury by comparing to etomidate (control), also an IV anesthetic with negligible effect on renal I/R injury,[12] in both normoglycemic and hyperglycemic conditions using a clinically relevant rat model of renal I/R. Primary endpoint was to investigate the reno-protective effect of propofol under hyperglycemic condition by histologic assessment of renal tubular damage. Secondary endpoints were to assess the associated protective mechanisms of propofol in terms of ROS production (superoxide dismutase, SOD), neutrophil infiltration (myeloperoxidase, MPO), concentrations of inflammatory cytokines (interleukin [IL]-1β, tumor necrosis factor [TNF]-α), iNOS activity, and NF-κB activation.

II. MATERIALS AND METHODS

1. Animals

This study was approved by the Committee for the Care and Use of Laboratory Animals at our hospital and was performed in accordance with the committee's Guidelines and Regulations for Animal Care. We studied Male Sprague-Dawley rats (10-12 weeks old, 250-300 g), after they had been acclimated for at least one week at the animal research centre of our hospital.

2. Study groups

Sixty male SD rats were randomly assigned to one of four experimental groups:

1). Normoglycemia-etomidate (NE) group (n=15): anesthesia was maintained by IV infusion of etomidate. After starting etomidate, normal saline was administered via a tail vein cannula.

2). Normoglycemia-propofol (NP) group (n=15): anesthesia was maintained with IV infusion of propofol. After starting propofol, normal saline was administered via a tail vein cannula.

3). Hyperglycemia-etomidate (GE) group (n=15): anesthesia was maintained with IV infusion of etomidate. After starting etomidate, dextrose (1.2 $g \cdot kg^{-1}$) was administered via a tail vein cannula.

4). Hyperglycemia-propofol (GP) group (n=15): anesthesia was maintained with IV infusion of propofol. After starting propofol, dextrose (1.2 $g \cdot kg^{-1}$) was administered via a tail vein cannula.

In each group, five sham rats were sacrificed 65 min after starting IV anesthetics without renal I/R injury, at which point kidneys and blood samples were collected (sham). The remaining rats in each group were killed 15 min (n=5) and 24 h (n=5) after renal I/R injury, at which point kidneys and blood samples were collected (Fig 1).

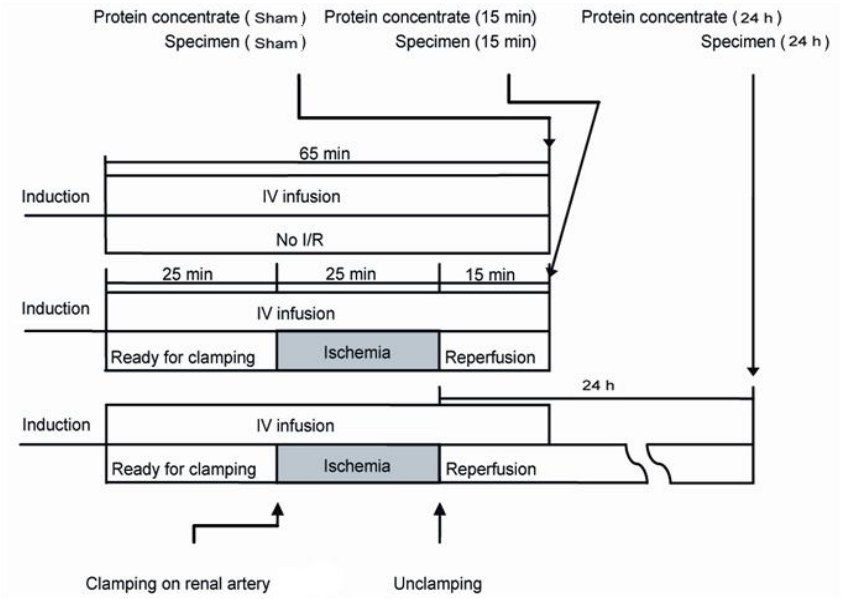


Figure 1. Study design and sampling time points.

In each group, five sham rats were sacrificed 65 min after starting IV anesthetics without renal I/R injury, at which point kidneys and blood samples were collected. The remaining rats in each group were sacrificed 15 min (n=5) and 24 h (n=5) after renal I/R injury, at which point kidneys and blood samples were collected. IV, intravenous; I/R, ischemia-reperfusion injury.

3. Surgical procedure and anesthesia

Anesthesia was induced in a chamber with 5% sevoflurane in oxygen (2 litre·min^{-1}) until the right and tail pinch reflexes were lost. Then the rats were transferred to a thermostatically controlled surgical table. A 24-G IV cannula was inserted into the tail vein and infusion of either propofol (Pofol; Dongkook Pharm Co., Jincheon, Korea) or etomidate (Etomidate-Lipuro; B. Braun Ltd, Melsungen, Germany) was initiated according to the group assignment. Propofol anesthesia began at 10 mg·kg^{-1}·h^{-1} and etomidate at 2 mg·kg^{-1}·h^{-1}, but these rates were adjusted according to the mean arterial pressure (MAP) and heart rate (HR). The rats were intubated with a 16-G IV cannula and artificially ventilated (Harvard Apparatus 683, USA) at 30-35 cycles·min^{-1} and a tidal volume of approximately 5 mL with 100% oxygen. Fifteen

minutes after starting IV anesthetics, dextrose (1.2 $g \cdot kg^{-1}$) or an equal volume of normal saline was administered using a tail vein cannula according to the group assignment. The right femoral artery was cannulated to monitor MAP and collect blood. HR was monitored by subcutaneous stainless steel electrodes connected to a PowerLab monitoring system (ML845 PowerLab with ML132; AD Instruments, USA). After preparation, rectal temperature was maintained at 37°C throughout the experiment.

The abdominal region was sterilised with a povidone iodine solution. Both kidneys were exposed through a midline incision, and the renal arteries and veins were dissected. Twenty-five minutes after surgical preparation, renal ischemia was induced by clamping both renal arteries with nontraumatic microvascular clamps for 25 min. Ischemia was confirmed by visually inspecting the kidney. After removing the clamps, we confirmed reperfusion, again by visual inspection. The rats were killed at the predefined time points to obtain kidneys and blood samples for further analyses. The abdomen was closed using a 3-0 vicryl running stitch on the muscular layer, and 4-0 silk for the skin layer. The wound was sterilised with povidone iodine solution. Antibiotics (30 $mg \cdot kg^{-1}$ ceftriaxone; CJ Pharm Co., Seoul, Korea) were given once intravenously after closing the abdomen. The animals were moved to their cage to recover with free access to water and food after they regained consciousness.

4. Measurement of HR, MAP, and blood sugar

HR and MAP were monitored during the procedures and were recorded 15 min after starting IV anesthetics (baseline), 15 min after clamping the renal arteries (during ischemia), and 15 min after removing the clamps (after reperfusion). A blood sugar concentration greater than 11.1 $mmol \cdot litre^{-1}$ was considered hyperglycemia. Blood sugar concentrations were monitored before administering dextrose (baseline), before inducing ischemia, and at the time when kidneys were removed.

5. Periodic Acid-Schiff assay

Both kidneys were cross-sectioned through the midpoint to measure histologic damage. The specimens were fixed in buffered formalin and embedded in paraffin. Pieces of kidney embedded in paraffin were cut into 4-μm sections and mounted on glass slides. Then, the sections were deparaffinised with xylene, counterstained with Periodic Acid-Schiff reagent, and examined under a light microscope (X200 magnification, Dialux 22; Leiz, Milan, Italy). A renal pathologist blinded to the study groups measured the histologic damage using the percentage of damaged tubules per field of view. Tubular damage was graded on a scale from 0 to 3: 0 = no damage, 1 = less than 25% tubular cell necrosis, 2 = 25-50% tubular cell necrosis, 3 = greater than 50% tubular cell necrosis.[14]

6. Preparation of protein

Protein extracted from cross-sectioned kidneys was used to analyze SOD, MPO, IL-1β, TNF-α, iNOS, phosphorylated-inhibitor of κB (I-κB) and phosphorylated NF-κB. Tissues were prepared as previously described.[15] Briefly, specimens were pulverised and dissolved in protein lysis buffer (PRO-PREP™ Protein Extraction Solution, iNtRON, Korea). The solution was homogenised with Pyrex Potter-Elvehjem Tissue Grinders (BLD Science, USA), centrifuged (12,000 × g for 30 min) at 4°C, and the supernatant was aliquoted and stored at –80 °C until analysis. Protein concentrations were determined using a Bradford protein assay kit (BioRad, USA).

7. Assay for superoxide dismutase activity

SOD activity in each protein sample was determined using a HT SOD kit according to the manufacturer's protocol (OXIS international, Portland, OR, USA).

8. Enzyme-linked immunosorbent assay (ELISA)

MPO concentrations were measured using a Rat MPO ELISA kit (Hycultbiotech,

Cell Sciences, WA, USA). TNF-α and IL-1β concentrations were measured using rat TNF-α and IL-1β assay kits (R&D, Minneapolis, MN, USA).

9. Western blot analysis

Equal amounts of protein were separated by SDS-PAGE and immunoblotted with iNOS (Abcam, Cambridge, MA, USA), phosphorylated-I-κB and phosphorylated-NF-κB (Cell Signalling Technology, Beverly, MA, USA) antibodies as previously described.[16] β-actin (Santa Cruz, CA, USA) antibody was used as a loading control. Protein concentrations were determined by densitometry values and normalized to β-actin.

10. Statistical analysis

The results are shown as mean (SD) and median (min-max). Data were analyzed by analysis of variance (ANOVA) with the Bonferroni *post-hoc* test for multiple comparisons and by Student's *t*-test as appropriate. The degree of renal tubular damage was compared with Kruskal-Wallis test with the Bonferroni *post-hoc* test for multiple comparisons. Statistical significance was defined as $P<0.05$. Data analysis was performed using SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA).

III. RESULTS

1. Physiologic variables

All rats after renal I/R injury survived without complications until the time of kidney harvesting. There were no differences in MAP and HR between groups throughout the study period (baseline, during ischemia, and after reperfusion), except baseline MAP which was lower in the NP than in the NE group (Table 1).

Table 1. Changes in hemodynamic parameters

			Baseline	During Ischemia	After Reperfusion
MAP	Normoglycemia	Etomidate	135 ± 11	123 ± 14	124 ± 16
		Propofol	115 ± 22*	119 ± 22	119 ± 35
	Hyperglycemia	Etomidate	131 ± 11	127 ± 16	129 ± 18
		Propofol	121 ± 17	116 ± 22	117 ± 36
HR	Normoglycemia	Etomidate	381 ± 41	391 ± 35	375 ± 21
		Propofol	357 ± 72	368 ± 67	361 ± 47
	Hyperglycemia	Etomidate	392 ± 39	384 ± 39	379 ± 33
		Propofol	361 ± 89	360 ± 63	355 ± 45

After reperfusion, MAP and HR monitored at 15 min after removal of the arterial clamping; Baseline, MAP and HR monitored at 15 min after starting IV anesthetics; During ischemia, MAP and HR monitored at 15 min after clamping renal artery and vein; HR, heart rate; MAP, mean arterial pressure. Values are presented as mean ± SD. * $P < 0.05$ compared to the Etomidate group.

Mean baseline blood sugar concentration of all rats was 5.8 (0.6) $mmol \cdot litre^{-1}$, and did not differ between groups. Before ischemia and 15 min after reperfusion, blood sugar concentrations were significantly higher in the hyperglycemic groups than in the normoglycemic groups though the differences disappeared at 24 h after reperfusion (Table 2).

Table 2. Time course of blood sugar concentrations

		Baseline	Before Ischemia	15 min	24 h
Normoglycemia	Etomidate	5.7 ± 0.7	5.6 ± 0.7	6.6 ± 0.8	5.6 ± 1.1
	Propofol	5.9 ± 0.6	5.8 ± 0.8	6.3 ± 0.8	5.9 ± 1.2
Hyperglycemia	Etomidate	5.7 ± 0.5	17.1 ± 1.2*†	18.1 ± 1.2*†	5.6 ± 0.8
	Propofol	5.7 ± 0.6	17.3 ± 1.1*†	17.8 ± 1*†	5.4 ± 0.8

Baseline, blood collected before administering dextrose; Before ischemia, blood collected before inducing ischemia; 15 min and 24 h, blood collected at 15 min and 24 h after reperfusion, respectively. Values are presented as mmol·litre^{-1} and mean ± SD. * $P < 0.05$ compared to the normoglycemia groups. † $P < 0.05$ compared to the baseline.

2. Histology

Compared to the sham groups, tubular damage at 24 h after reperfusion was significantly greater in all the etomidate and propofol groups. Tubular damage was significantly greater under hyperglycemia than under normoglycemia at 24 h after reperfusion in the etomidate group, while in the propofol group, tubular damage did not differ regardless of their glycemic conditions. Under hyperglycemia, tubular damage was greater in the etomidate group than in the propofol group at 24 h after reperfusion (Fig 2, Table 3).

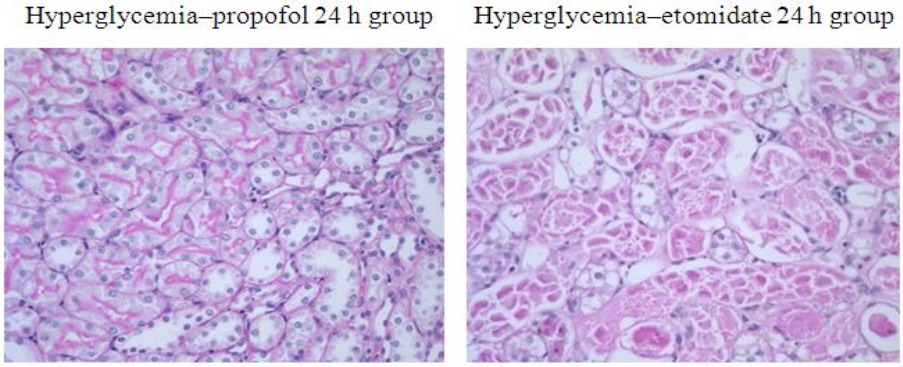


Figure 2. Representative histology of renal tubules in the hyperglycemia–propofol and hyperglycemia–etomidate rats sacrificed 24 h after ischemia-reperfusion injury.

Table 3. Damaged grade of renal tubules

		Sham	15 min	24 h
Normoglycemia	Etomidate	0 (0-0)	0 (0-1)	1 (1-2)†
	Propofol	0 (0-0)	0 (0-1)	1 (0-2)†
Hyperglycemia	Etomidate	0 (0-0)	0 (0-1)	2 (1-3)†‡
	Propofol	0 (0-0)	0 (0-0)	1 (0-2)*†

Graded damage (20 X 20 μm; magnification × 400) of renal tubules in rats sacrificed at various time points after ischemia-reperfusion injury. Sham, specimens collected 65 min after starting IV anesthetics without renal ischemia-reperfusion injury; 15 min and 24 h, specimens collected 15 min and 24 h after reperfusion, respectively. Values are presented as median (min-max). $^{*}P < 0.05$ compared to the etomidate groups. $^{\dagger}P < 0.05$ compared to the each corresponding value of the sham groups. $^{\ddagger}P < 0.05$ compared to the normoglycemia groups.

3. ROS production

After reperfusion, SOD activities decreased significantly in the NE, NP and GE groups compared to each corresponding value of the sham group, while it was unchanged in the GP group. In the propofol groups, SOD activities were significantly higher under hyperglycemia than under normoglycemia at 15 min and 24 h after reperfusion. SOD activities were significantly higher in the propofol groups than in the etomidate groups after reperfusion under both normo- and hyperglycemic conditions (Fig 3A).

4. Neutrophil infiltration

After reperfusion, the MPO concentrations increased significantly in the etomidate groups compared to each corresponding value of the sham groups, while they were unchanged in the propofol groups. In the etomidate groups, the MPO concentrations were significantly higher under hyperglycemia compared to normoglycemia at 24 h after reperfusion. The MPO concentrations were significantly lower in the propofol groups than in the etomidate groups after reperfusion under both normo- and hyperglycemic conditions (Fig 3B).

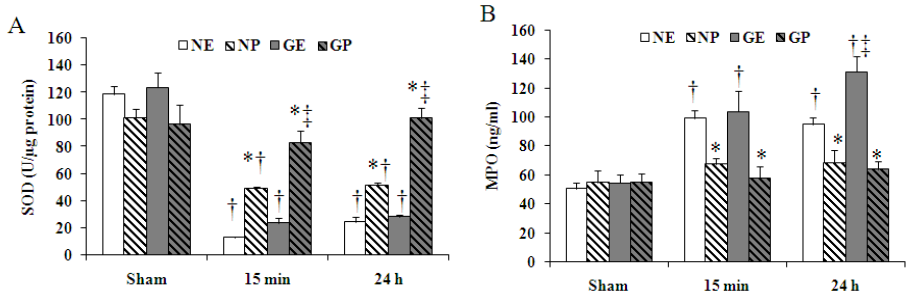


Figure 3. Superoxide dismutase (A) and myeloperoxidase (B) levels in renal tissue after ischemia-reperfusion injury. Sham, protein collected 65 min after starting IV anesthetics without renal ischemia-reperfusion injury; 15 min and 24 h, protein collected 15 min and 24 h after reperfusion, respectively; SOD, superoxide dismutase; MPO, myeloperoxidase; NE, normoglycemia–etomidate; NP, normoglycemia–propofol; GE, hyperglycemia–etomidate; GP, hyperglycemia–propofol. Values are presented as mean (SD). $^{*}P < 0.05$ compared to the etomidate groups. $^{\dagger}P < 0.05$ compared to the each corresponding value of the sham groups. $^{\ddagger}P < 0.05$ compared to the normoglycemia groups.

5. Inflammatory cytokines

In the etomidate groups, the IL-1β and TNF-α concentration increased significantly after reperfusion compared to each corresponding value of the sham group, while they were comparable in the propofol groups regardless of their

glycemic conditions. In the etomidate groups, the IL-1β and TNF-α concentration were significantly higher under hyperglycemia than under normoglycemia after reperfusion, while there were no significant differences within the propofol groups. Under hyperglycemia, the IL-1β and TNF-α concentration were significantly lower in the propofol groups than in the etomidate groups after reperfusion (Fig 4).

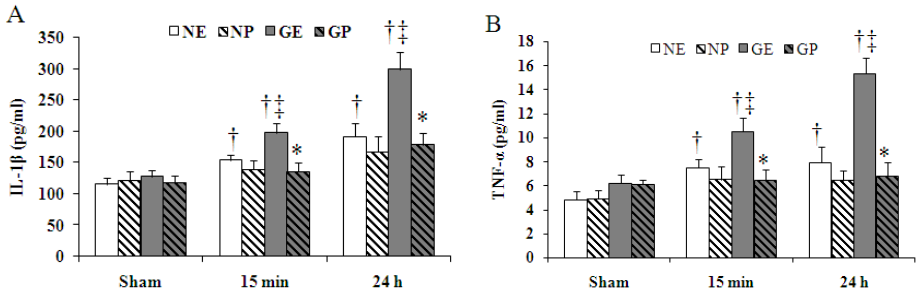


Figure 4. IL-1β (A) and TNF-α (B) levels in renal tissue after ischemia-reperfusion injury. Sham, protein concentrate collected 65 min after starting IV anesthetics without renal ischemia-reperfusion injury; 15 min and 24 h, protein collected 15 min and 24 h after reperfusion, respectively; ; IL-1β, Interleukin-1β; TNF-α, tumour necrosis factor-α; NE, normoglycemia–etomidate; NP, normoglycemia–propofol; GE, hyperglycemia–etomidate; GP, hyperglycemia–propofol. Values are presented as mean (SD). $^{*}P < 0.05$ compared to the etomidate groups. $^{\dagger}P < 0.05$ compared to the each corresponding value of the sham groups. $^{\ddagger}P < 0.05$ compared to the normoglycemia groups.

6. iNOS activity

Even though there were no I/R, iNOS production of the sham group was significantly higher under hyperglycemia than under normoglycemia. After reperfusion, iNOS production increased significantly in all groups compared to each corresponding value of the sham group, except in the GP group at 24 h after reperfusion. In the etomidate groups, iNOS production was significantly higher

under hyperglycemia than normoglycemia, while it was significantly lower under hyperglycemia than normoglycemia in the propofol groups at 15 min after reperfusion. iNOS production was significantly lower in the propofol groups compared to the etomidate groups regardless of their glycemic conditions at 15 minutes after reperfusion. However, at 24 h after reperfusion, it was significantly lower in the propofol group only under hyperglycemia (Fig 5).

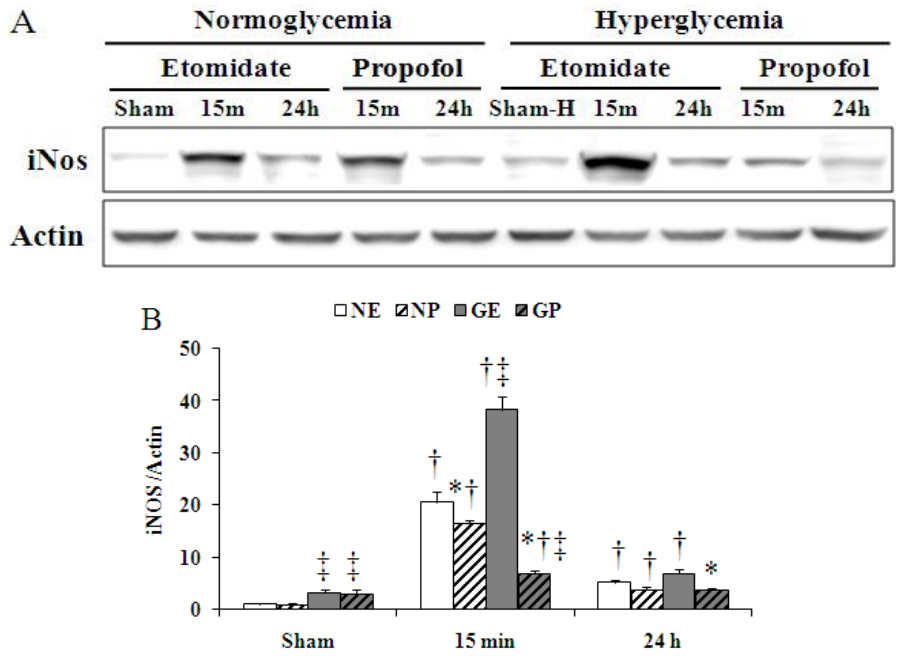
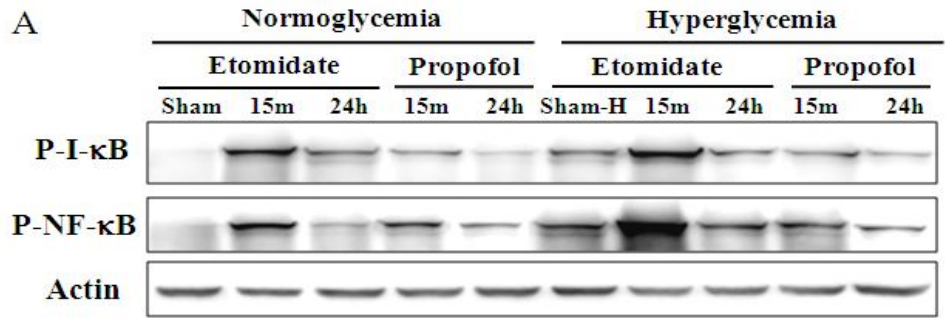


Figure 5. Representative Western blots showing iNOS expression in renal tissue (A). Expression of iNOS in renal tissue after ischemia-reperfusion injury (B). 15 min and 24 h, protein collected 15 min and 24 h after reperfusion, respectively; iNOS, inducible nitric oxide synthase; NE, normoglycemia–etomidate; NP, normoglycemia–propofol. GE, hyperglycemia–etomidate; GP, hyperglycemia–propofol. Values are presented as mean (SD). $^{*}P < 0.05$ compared to the etomidate groups. $^{\dagger}P < 0.05$ compared to the each corresponding value of the sham groups. $^{\ddagger}P < 0.05$ compared to the normoglycemia groups.

7. NF-κB activation

Even though there were no I/R, the phosphorylations of I-κB and NF-κB of the sham group were significantly higher under hyperglycemia than under normoglycemia. At 15 min after reperfusion, the phosphorylation of I-κB significantly increased in all groups compared to each corresponding value of the sham group except in the GP group. At 24 h after reperfusion, the phosphorylation of I-κB remained significantly increased compared to each corresponding value of the sham group in the NE and NP group, whereas it was similar and significantly lower in the GE and GP group, respectively. At 15 min after reperfusion, the phosphorylation of NF-κB was significantly increased in all groups compared to each corresponding value of the sham group except in the GP group. At 24 h after reperfusion, only the phosphorylation of NF-κB of the GP group was significantly lower compared to the corresponding value of the sham group. In the etomidate groups, the phosphorylations of I-κB and NF-κB were significantly higher under hyperglycemia than under normoglycemia except that of I-κB at 24 h after reperfusion, while there were no significant changes within the propofol groups. The phosphorylations of I-κB and NF-κB were significantly lower in the propofol groups than in the etomidate groups regardless of their glycemic conditions except that of NF-κB at 24 h after reperfusion under normoglycemia (Fig 6).



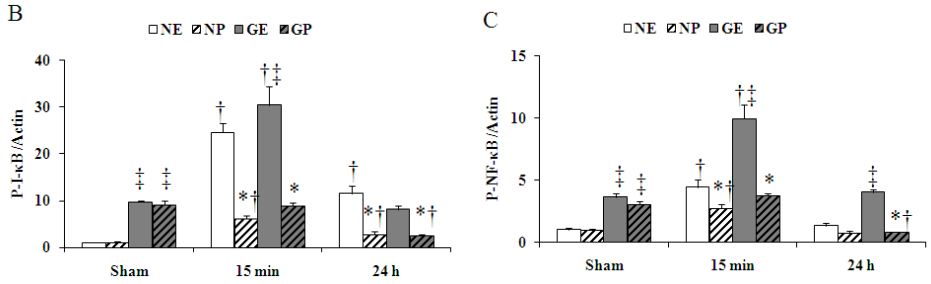


Figure 6. Representative Western blots showing phosphorylated-I-κB, and phosphorylated-NF-κB expression in renal tissue (A). Expression of phosphorylated-I-κB (B) and phosphorylated-NF-κB (C) in renal tissue after ischemia-reperfusion injury. 15 min and 24 h, protein collected 15 min and 24 h after reperfusion, respectively; I-κB, inhibitor of κB; NF-κB, nuclear transcriptional factor-κB; NE, normoglycemia–etomidate; NP, normoglycemia–propofol. GE, hyperglycemia–etomidate; GP, hyperglycemia–propofol. Values are presented as mean (SD). $^{*}P < 0.05$ compared to the etomidate groups. $^{\dagger}P < 0.05$ compared to the each corresponding value of the sham groups. $^{\ddagger}P < 0.05$ compared to the normoglycemia groups.

IV. DISCUSSION

The purpose of the present study was to evaluate whether propofol anesthesia would reduce renal injury under hyperglycemic as well as normoglycemic conditions in a clinically relevant rat model of renal I/R injury induced by renal artery occlusion. In the current study, propofol conveyed significant reno-protective effect against I/R injury in the presence of hyperglycemia as evidenced by histologic evaluation of renal tubular damage. This finding was accompanied by preserved antioxidant ability, attenuated neutrophil infiltration, proinflammatory cytokine and iNOS production, and down-regulation of the phosphorylation of I-κB and NF-κB.

Hyperglycemia is undoubtedly one of the major risk factors associated with adverse outcome in surgical patients. Recently, even transient hyperglycemic episodes during I/R in non-diabetic animals have been reported to significantly intensify renal injury through various mechanisms including ROS production and activation of systemic inflammatory reactions.[7,8] Indeed, hyperglycemia has been shown to mitigate the protective effect of volatile anesthetics against I/R injury.[17] Propofol is a widely used IV anesthetic agent with a proven antioxidant activity similar to vitamin E.[9] Furthermore, propofol has been demonstrated to modulate inflammatory reaction and oxidative stress through attenuating iNOS activity and NF-κB expression in cardiac surgical patients.[10,11] Although its reno-protective effect under hyperglycemia has not been validated heretofore, propofol could attenuate edema formation and lactate accumulation in a hyperglycemic cerebral I/R injury model.[13] Based on these theoretical advantages of propofol, we firstly planed to investigate the reno-protective effect of propofol in a hyperglycemic rat model through histologic assessment of renal tubular damage. In addition, we also assessed the activation of harmful biochemical markers related to the pathogenesis of renal I/R injury, which only have been demonstrated to be modulated by propofol under normoglycemic condition.[10,11]

The first-line defense mechanisms after reperfusion include antioxidants such as

SOD, catalase and glutathione dismutase. SOD is known as the most important endogenous antioxidant defense mechanism against oxidative stress,[18] which is rapidly depleted by accumulating ROS during I/R.[1] Several studies reported that antioxidant therapy such as beta carotene[19] and curcumin[20] before renal I/R injury preserved SOD activity measured at 4 h and 24 h after reperfusion, respectively, in normoglycemic condition.

The MPO activity is widely used to quantify the number of neutrophils in a tissue and serves as a good indicator of neutrophil infiltration.[21] Increased MPO activity following I/R was reported to indicate renal injury associated with free radical formation,[22] and the recruitment of neutrophil was known as the major form of death after renal I/R injury.[23] Activation of neutrophils and macrophages contribute to control an active inflammatory process after I/R. This process is associated with rapid release of proinflammatory cytokines such as IL-1, IL-6, TNF-α and IL-8.[24,25] Tethering and adherence of neutrophils and macrophages to endothelial surface is controlled by chemoattractant chemokines and up-regulation of intracellular adhesion molecules (ICAM).[26] Previous study showed that functional blockage of IL-1 or TNF-α attenuated I/R injury, which was associated with reduced expression of ICAM-1.[27]

The contribution of NO in the pathogenesis of renal I/R injury is unclear, however, several studies have shown that NO produced from iNOS is cytotoxic to renal tubules, especially at supraphysiological concentrations.[28,29] Moreover, during I/R, NO reacts rapidly with O^{2-} and produces peroxinitrate anion which is a potent oxidant.[30] Peroxinitrate anion also potentiates inflammation by inactivating SOD protein through nitration of its tyrosin residue.[30] In conjunction, selective inhibition of iNOS during I/R could reduce the degree of renal I/R injury.[31]

NF-κB, a transcription factor, plays a pivotal role in inflammation and oxidative stress during I/R.[32] NF-κB activation is tightly regulated by I-κB proteins which complex with NF-κB in the cytoplasm. Various stimuli relevant to renal injury such as proinflammatory cytokines, oxidative stress, immune mediator, and advanced glycosylation end product under chronic hyperglycemia activate NF-κB.[33]

Activating stimuli facilitate phosphorylation of I-κB leading to release of I-κB-bound NF-κB, which then translocates into the nucleus and stimulates gene transcription.[34] NF-κB is also a central mediator in inflammatory process. Cytokines (TNF-α, IL-1, IL-6), adhesion molecules (ICAM-1, VCAM-1), and enzymes (iNOS) are known as proinflammatory NF-κB target gene.[35] Moreover, SOD is also known as a target gene of NF-κB.[34] Thus, a considerable cross-link exists between the assessed biochemical markers in the current study affecting their fate and role in the pathogenesis of renal I/R injury.

As our results indicate, transient hyperglycemia present during renal I/R aggravated renal tubular damage and resulted in increases of neutrophil infiltration at 24 h after reperfusion, concentrations of proinflammatory cytokines at 15 min and 24 h after reperfusion, and iNOS production and NF-κB activation at 15 min after reperfusion under etomidate (control) anesthesia, which is in agreement with the findings of a previous study.[7] In contrast, propofol anesthesia provided significant reno-protection in terms of renal tubular damage in the presence of hyperglycemia. Moreover, propofol was able to modulate all of the assessed biochemical markers associated with the pathogenesis of renal I/R injury regardless of the glycemic condition. In detail, propofol preserved SOD activity from the early reperfusion period on, which was even more prominent under hyperglycemic condition. Propofol attenuated the increase in MPO activity, as well as the increase in the concentrations of IL-1β and TNF-α under both normo-and hyperglycemic conditions. Propofol also attenuated iNOS production, which was even more prominent under hyperglycemic condition, and phosphorylation of I-κB and NF-κB regardless of the glycemic conditions.

Considering the above mentioned existence of a cross-talk between the biochemical markers and the blood concentration-time profile of propofol infusion,[35] these protective effects of propofol at 24 h after reperfusion may be associated with decreased NF-κB activation at acute period after reperfusion. Attenuation of NF-κB activation under propofol anesthesia may cause resultant reduction in MPO, IL-1, TNF-α production and iNOS production, and preserved

SOD activity at 24 h after reperfusion. Taken together, the results of the current study provide primary evidence regarding the role of propofol as a reno-protective strategy even in hypeglycemic conditions. Although species difference exist regarding the susceptibility to I/R injury, our results pose significant clinical implication considering that the dose of propofol and renal I/R model used in this study mimic the current clinical situations,[36,37] and that hyperglycemia is frequently encountered in the cardiovascular surgical theatre.

Of interest, phosphorylation of I-κB and NF-κB, and production of iNOS were significantly higher in the sham-hyperglycemia group compared to sham-normoglycemia group in the current study, which were further increased by I/R. Although the role of NF-κB and iNOS in transient hyperglycemia without I/R has not been known, chronic hyperglycemia was reported to be closely associated with impaired NO availability[5] and activation of NF-κB.[33] These findings implicate that transient hyperglycemia without I/R facilitates activation of NF-κB and production of iNOS, which may serve as important modulatory targets of propofol in attenuating the renal I/R injury in the presence of hyperglycemia.

The limitation of this study is as follows. For clinical relevance, we gave ischemic insult for 25 min because median renal ischemia time during juxtarenal aortic aneurysm repair was 27 min.[37] However, the ischemia model may not have been injurious enough to validate clear differences in renal tubular damage under normoglycemic condition. In contrast, as hyperglycemia itself aggravated I/R injury, validation of propofol's reno-protective effect against renal I/R injury proved to be feasible and more definite.

V. CONCLUSION

Propofol anesthesia conferred beneficial influence against renal I/R injury aggravated by hyperglycemia, as confirmed by attenuation of tubular cell death. This protection was mediated by propofol maintaining SOD activity, while mitigating neutrophil infiltration, proinflammatory cytokine activation, iNOS production, and NF-κB activation, which were more prominent under hyperglycemic condition.

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ABSTRACT (IN KOREAN)

Propofol이 고혈당에서 신장의 허혈/재관류 손상에 미치는 영향

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고혈당은 신장의 허혈/재관류 시에 산소 유리기의 발생과 염증 반응을 증가시킴으로써 신 손상을 악화시킨다고 알려져 있다. 본 연구는 항산화 작용이 있다고 알려져 있는 propofol 을 이용한 마취가 정상혈당에서뿐만 아니라 고혈당 하에서도 신장의 허혈/재관류 시에 신 보호 효과가 있는 지 확인하고자 수행되었다.

10-12 주령의 백서 60 마리를 정상혈당-etomidate 군, 정상혈당-propofol 군, 고혈당-etomidate 군, 고혈당-propofol 군의 네 군으로 나누어 군에 따라서 etomidate 혹은 propofol로 마취를 시행하였고 또한 kg 당 1.2 g 의 dextrose 혹은 동량의 생리식염수를 마취 후 정맥 주입하여 고혈당 군에서는 일시적인 고혈당을 유발하였다. 각 군마다 5 마리씩 허혈/재관류 손상을 주지 않고 마취 후 65 분이 지난 다음 신장을 적출하였고 나머지 백서들은 신 동맥을 결찰하여 25 분간 허혈을 유발한 후 각 군마다 5 마리씩 15 분과 24 시간 동안 재관류시킨 후 신장을 적출하였다. 적출한 신

조직을 이용하여 각 군마다 시간 별로 조직학적인 손상 정도와 염증 반응과 관련된 지표들을 비교하였다.

고혈당 하에서 propofol로 마취를 시행한 경우 etomidate 마취와 비교하여 재관류 24 시간 후에 신 손상이 유의하게 감소하였으며, etomidate 마취 하에서는 정상혈당에서보다 고혈당에서 신 손상이 유의하게 증가하였으나 propofol 마취 하에서는 혈당에 따른 신 손상의 증가를 보이지 않았다. 또한 재관류 15 분 후와 24 시간 후 모두에서 propofol 마취 하에서는 etomidate 마취와 비교하여 항산화 방어기제인 superoxide dismutase가 유지되었고 myeloperoxidase, interlukin-1β, tumor necrotic factor-α, inducible nitric oxide synthase 가 덜 증가하는 경향을 보였으며 이는 고혈당 하에서 더욱 명확하게 나타났다. 재관류 15분 후에 propofol 마취 하에서는 etomidate 마취와 비교하여 inhibitor of κB 와 nuclear factor-κB 의 인산화 반응 역시 유의하게 억제되었으며 이는 재관류 24 시간 후에도 유지되는 경향을 보였다.

결론적으로 propofol은 고혈당 하에서 신장의 허혈/재관류 손상 시 신 보호 효과를 나타내었으며 이는 항산화 방어기제를 유지시키고 염증 반응의 진행을 억제하는 propofol의 작용과 관련이 있다고 생각된다.

핵심되는 말: 고혈당, 신 보호 효과, 허혈/재관류 손상, propofol