ORIGINAL ARTICLE

Preventive effects of propofol and ketamine on renal injury in unilateral ureteral obstruction

**Bayazit Dikmen · Hatice Yagmurdur ·
Turgay Akgul · Muzeyyen Astarci ·
Huseyin Ustun · Cankon Germiyanoglu**

Abstract

Purpose The aim of the present study was to investigate the preventive effects of propofol and ketamine as anesthetics on renal injury in unilateral ureteral obstruction (UO).

Methods Twenty-four male New Zealand white rabbits were randomly assigned to four groups of six rabbits each. Anesthesia was induced and maintained with propofol in groups 1 and 2 and with ketamine in groups 3 and 4. Groups 2 and 4 received complete left ureteral ligation. Groups 1 and 3 (control groups) underwent an identical surgical procedure without ureteral ligation. At 14 days of obstruction, animals were sacrificed and ipsilateral kidneys were removed for determination of tissue nitric oxide (NO) levels and immunohistochemical evaluation of endothelial nitric oxide synthase (eNOS), inducible NOS (iNOS), and apoptosis protease-activating factor 1 (APAF-1).

Results Between groups 1 and 3, there were no differences in tissue NO levels and eNOS, iNOS, and APAF-1 expressions. iNOS and APAF-1 expressions were at the mild to moderate levels in group 2, but these parameters were markedly increased in group 4 at 14 days of obstruction. Also, elevated expression of iNOS was accompanied by a high NO production in group 4 compared with group 2. Although eNOS expressions were increased in both groups 2 and 4, there were no significant differences between these groups.

Conclusions Propofol as an anesthetic agent may attenuate NO-induced renal tubular cell apoptosis by down-regulating the expression of iNOS in an animal model of unilateral UO.

Keywords Propofol · Ketamine · Kidney · Ureteral obstruction · Nitric oxide · Nitric oxide synthase · Apoptosis

B. Dikmen · H. Yagmurdur
Clinic of Anesthesiology and Reanimation,
The Ministry of Health Ankara Research and Training Hospital,
Ankara, Turkey

T. Akgul · C. Germiyanoglu
Clinic of Urology, The Ministry of Health Ankara
Research and Training Hospital, Ankara, Turkey

M. Astarci · H. Ustun
Department of Pathology, The Ministry of Health Ankara
Research and Training Hospital, Ankara, Turkey

H. Yagmurdur (✉)
Esat Cad 102/10, Kucukesat, Cankaya,
06660 Ankara, Turkey
e-mail: hyagmurdur@yahoo.com; hyagmurdur@gmail.com

Introduction

Ureteral obstruction (UO) is a disease process that may result from a diversity of congenital or acquired conditions, ranging from calculi to strictures and to inflammatory processes and malignancies. The clinical presentation depends on the speed of onset and whether obstruction is complete or incomplete. Sustained unilateral UO can eventually leads to tubulointerstitial fibrosis, especially in cases of complete obstruction [1]. Recently, it has been demonstrated that urinary obstruction mediates progressive apoptosis of both renal tubular and interstitial cells [2]. A number of different stimuli in the obstructed kidney may contribute to apoptosis, including mechanical stretch of tubular cells [3], oxidative stress [4], as well as production of proapoptotic factors such as nitric oxide (NO) [5].

NO, a freely diffusible, water- and lipid-soluble gaseous molecule with a short half-life, is formed from L-arginine and molecular oxygen by a family of nitric oxide synthases (NOS) [6]. NOS has been identified in several tissues; however, only three isoforms—neural NOS (nNOS), inducible macrophage NOS (iNOS), and endothelial NOS (eNOS)—have been identified, with the eNOS and iNOS forms being involved in renal vascular responses [7, 8].

Propofol (2,6-diisopropyl phenol), a highly lipid-soluble anesthetic, has a potent antioxidant activity against lipid peroxidation in both in vitro and in vivo studies [9, 10]. Propofol also attenuates ischemia–reperfusion-induced lipid peroxidation in humans [11, 12]. The effect of ketamine on oxidative stress is likely complicated. Ketamine has a scavenging effect and an effect to suppress reactive oxygen species production by neutrophils [13]. On the other hand, ketamine reportedly increases reactive oxygen species in the brain [14].

We previously showed the involvement of propofol in the prevention of NO-induced germ cell-specific apoptosis through downregulation of iNOS expression in an animal model of testicular torsion and detorsion [15, 16]. The aim of the present study was to investigate the preventive effects of propofol and ketamine as anesthetics on UO-induced renal injury with renal expressions of iNOS, eNOS, and renal tubular apoptosis in the ipsilateral kidney after a complete unilateral UO experimentally.

Methods

Animals

The experimental protocol used in this study was approved by the Animal Investigation Committee of the Ministry of Health Ankara Research and Training Hospital and adhered to National Institutes of Health guidelines for the use of experimental animals (protocol approval no: EPKK-0309-2249). Animals were housed in individual cages in a cohorted temperature-controlled room with alternating 12 h light/dark cycles, and acclimated for a week before the study. Food was removed 6 h before the study, but all animals were allowed free access to water. Twenty-four male New Zealand white rabbits, weighing between 1800

and 2000 g, were randomly assigned into four groups with six rabbits in each (Table 1).

Anesthesia

Anesthesia was induced with 8 mg kg^{-1} propofol (Abbott Propofol, Abbott Laboratories, Chicago, IL) in groups 1 and 2 and with 10 mg kg^{-1} ketamine (Ketalar, Parke-Davis, EWL Eczacibasi Warner Lambert, Istanbul, Turkey) in groups 3 and 4 via the left marginal ear vein. Then a 24-gauge catheter was placed in the right lateral ear vein to maintain the anesthesia and fluids. Animals then underwent aseptically placement of left ear arterial line with polyethylene tubing to continuously monitor the mean arterial pressure (MAP). Anesthesia was maintained with 20 mg kg^{-1} h^{-1} propofol in groups 1 and 2 and 25 mg kg^{-1} h^{-1} ketamine in groups 3 and 4 during the experiment. After a baseline (before surgical incision) measurement of MAP was obtained, it was monitored during the experiment. Duration of anesthesia was approximately 90 min from the induction to the end of experiment.

Surgical procedure

After induction of anesthesia they were placed on a heated table to maintain rectal temperature at 37−38°C. The depth of surgical anesthesia was similar between groups. This was assessed by checking the interdigital (pedal) reflex using surgical forceps. This reflex was absent from both fore and hind legs. Through a midline abdominal incision, the left ureter was exposed and completely ligated around its midsection with a 5–0 silk in groups 2 and 4. In the control groups (groups 1 and 3), animals underwent an identical surgical procedure without ureteral ligation. After each surgical intervention, the incision was closed in layers. Then, we pulled out the arterial line and applied plastic clip on the top of the cotton ball for prevention of further bleeding. At the end of experiment, animals were allowed to recover from the anesthesia and allowed food and water ad libitum. At 14 days of complete obstruction, the animals in groups 1, 2 and 3, 4 were sacrificed by a propofol and ketamine overdose, respectively, and ipsilateral kidneys were removed for biochemical analysis and pathological evaluation.

Biochemical analysis

Determination of tissue NO levels

Because the direct measurement of NO is very difficult in biological specimens, tissue NO production in the kidney was quantified indirectly as nitrite (NO_2^-)/nitrate (NO_3^-) from kidney homogenates based on the Griess method,

Table 1 Experimental conditions and animal groups

Group	Conditions	n
1	Propofol + no obstruction	6
2	Propofol + obstruction	6
3	Ketamine + no obstruction	6
4	Ketamine + obstruction	6

using 2–10 μM sodium nitrite or sodium nitrate as the standards [17]. The data in this study represent the sum of nitrite and nitrate, and were expressed as micromoles of NO per gram of wet tissue (μmol g^{-1}).

Pathological evaluation

Immunohistochemistry for eNOS, iNOS, and APAF-1

Samples were embedded in paraffin, cut into 5 μm sections, and prepared for immunohistochemical staining. Specimens were evaluated by independent pathologists in a blind and randomly numbered fashion. All sections were applied to poly-L-lysine-coated slides and dewaxed in xylene. After washing in a decreasing series of ethanol and water, sections were treated with cold 3% hydrogen peroxide (H$_2$O$_2$) in distilled water for 30 min to block endogenous peroxidases. Sections were washed with phosphate-buffered saline (PBS) and incubated overnight at 4°C with an eNOS monoclonal, an iNOS polyclonal (Lab Vision Corp., Neomarkers, Fremont, CA), or an apoptosis protease-activating factor 1 (APAF-1) antibody (Lab Vision Corp., Neomarkers, Fremont, CA) at a dilution of 1/200 in PBS for the NOS antibodies and 1/100 for APAF-1, for 16 h at 4°C. The sections were incubated with biotinylated immunoglobulins for the corresponding primary antibody. Sections were washed with PBS three times and incubated with an avidin–biotin–peroxidase complex. Then, the peroxides were visualized by incubating the sections in diaminobenzidine and H$_2$O$_2$. Negative controls were treated identically by omitting the incubation with the primary antibody. Immunohistochemical staining was evaluated semiquantitatively using an additive immunoreactive score reflecting signal intensity, that is 0, negative; 1, mild; 2, moderate and 3, marked; and the number of immunopositive cells, that is 0, no positive cells; 1, less than 10% positive cells; 2–10% to 50% positive cells, and 3, greater than 50% positive cells.

Statistical analysis

Where appropriate, the data were expressed as the mean ± SD. Quantitative analysis of NO, iNOS, eNOS, and APAF-1 was performed with the Kruskall–Wallis one-way analysis of variance (ANOVA) test among groups. Paired comparisons of groups were made with the Mann–Whitney U test for assessment of which group or groups were the reasons for the difference. Then, the $\alpha = 0.05$ error level was divided into the number of comparisons and Bonferroni's corrections made. Immunohistochemistry for eNOS, iNOS, and APAF-1 was evaluated qualitatively by χ^2 analysis with Yates' correction factor. p values <0.05 were considered to be significant.

Table 2 NO levels of ipsilateral kidneys

Group	NO (μmol g^{-1})
1	2.5 ± 1.8
2	5.1 ± 1.1*
3	2.2 ± 1.3
4	9.8 ± 1.6**,†

Values are mean ± SD

NO Nitric oxide

*$p < 0.05$ vs. group 1, **$p < 0.05$ vs. group 3, †$p < 0.05$ vs. group 2

Results

The baseline (before surgical incision) MAP were 94 ± 15 mmHg in group 1, 90 ± 12 mmHg in group 2, 96 ± 10 mmHg in group 3, and 95 ± 13 mmHg in group 4. There were no significant differences in baseline measurement of MAP between the groups ($p > 0.05$). Then the MAP was maintained within 10% of baseline levels by the adjustment of infusion of intravenous fluid in all animals during experiment. Also there was no difference in the volume of fluid needed in all groups ($p > 0.05$).

Table 2 shows the ipsilateral kidney NO values for all groups. Although complete UO in group 2 caused significantly increased levels of NO compared with group 1 ($p = 0.008$), obstruction in group 4 caused a further increase in NO levels compared with groups 3 and 2 ($p = 0.001$ and $p = 0.007$, respectively). There were no significant differences in NO levels between groups 1 and 3.

Immunohistochemical analysis indicated that eNOS and iNOS expressions were mild in the control groups and not different between groups (groups 1 and 3) (Figs. 1a, 3a, 2, 4) ($p > 0.05$). eNOS expression in rabbits undergoing complete UO increased significantly compared with control sections at 14 days of obstruction ($p < 0.05$). This tendency was maintained without significant differences between groups 2 and 4 (Figs. 1b, c, 2) ($p > 0.05$).

At 14 days of complete UO, marked iNOS expression was observed in the ipsilateral kidneys in group 4, mainly in the tubular cells from renal cortex (Figs. 3c, 4) ($p < 0.05$). Also, elevated expression of iNOS was accompanied by a high NO production in group 4. However, iNOS reactivity was mild to moderate in rabbits undergoing complete UO under propofol anesthesia (Figs. 3b, 4) ($p < 0.05$).

Tissue sections from kidneys of control animals stained immunohistochemically for APAF-1 revealed very few stained nuclei (Figs. 5a, 6). Remarkably increased numbers of apoptotic cells were detected in group 4, but only mild levels of apoptotic cells were
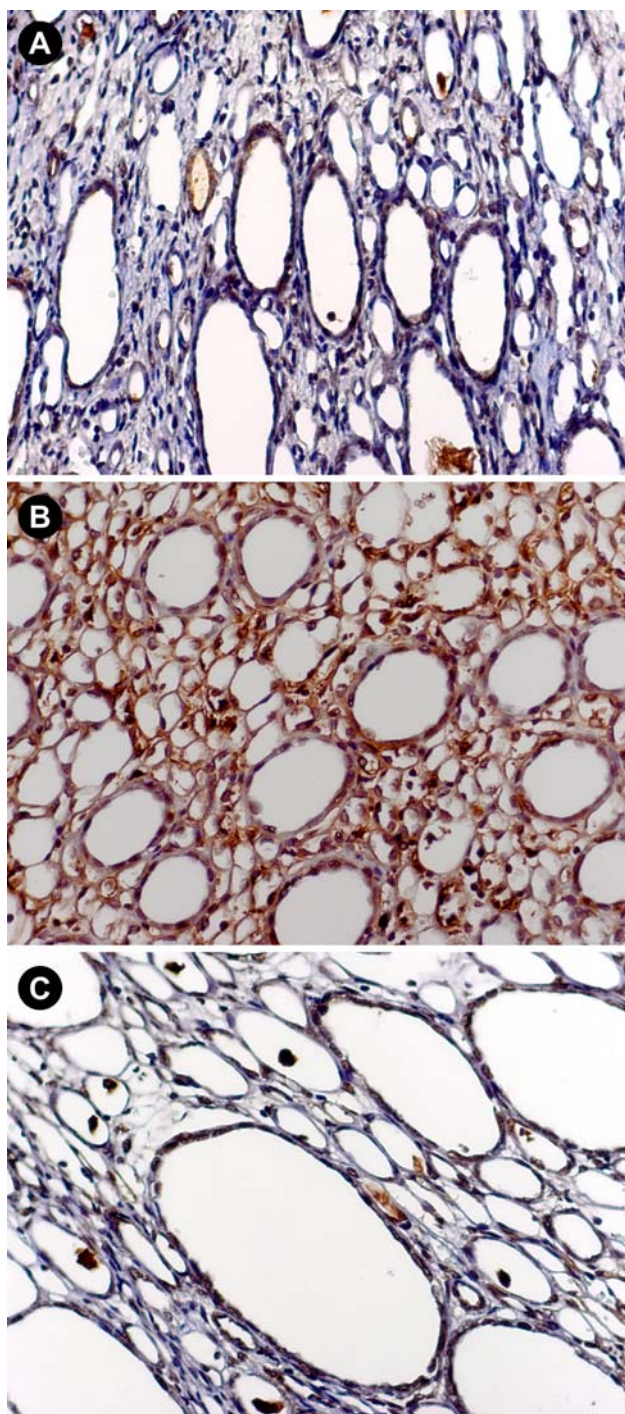
Fig. 1 Immunohistochemical analysis showing representative expression of endothelial nitric oxide synthase (eNOS) at 14 days of ureteral obstruction. **a** Mild eNOS immunostaining is present in the control groups (1 and 3); **b** moderate eNOS immunostaining is present in group 2; **c** moderate eNOS immunostaining is present in group 4. Magnification, ×400

present in rabbits undergoing complete UO under propofol anesthesia at 14 days after obstruction (Figs. 5b, c, 6) ($p < 0.05$).
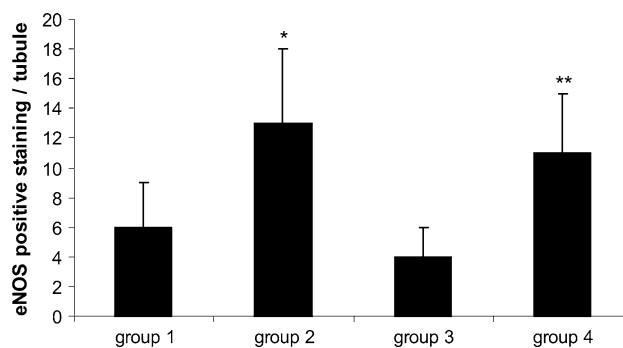

Fig. 2 Number of immunopositive cells for endothelial nitric oxide synthase (eNOS) per renal tubule. eNOS expression in animals undergoing complete UO increased significantly compared with control sections at 14 days of obstruction. This tendency was maintained without significant differences between groups 2 and 4. *$p < 0.05$ vs. group 1, **$p < 0.05$ vs. group 3. Data are presented as mean ± SD

Discussion

The most significant finding of the present study is that complete UO for 14 days strikingly induces iNOS that elevates NO production and renal tubular apoptosis in the model of complete UO of the rabbit. Propofol significantly reduced the NO level and APAF-1 expression as a marker of the apoptotic index by inhibition of iNOS expression in the kidney at 14 days of complete UO.

The changes in intrarenal pressure could lead to tubular cell stretch in complete UO [18]. Several reports have shown that stretch induces NO production [19, 20]. In the kidney, many types of cells are capable of secreting NO. However, the changes in intrarenal pressure accompanying UO could result in activation of tubular NOS [21]. Although the cytotoxic actions of excessive NO production may be involved in several potential mechanisms [22], there is more evidence that the toxicity of NO is primarily mediated by the highly cytotoxic agent peroxynitrite [23]. Large amounts of NO produced by iNOS react rapidly with the superoxide radical to form peroxynitrite. Peroxynitrite has been proposed to play a significant role in cellular damage by initiation of the lipid peroxidation process. Peroxynitrite is also involved in the nitration of tyrosyl residues in proteins; this reaction can alter cell-signaling processes and plays a pivotal role in the mechanism of apoptosis [24].

In the kidney, eNOS is found in the endothelial cells of the renal arterioles, glomeruli and peritubular capillaries. iNOS is found in mesangial and tubular cells, as well as in infiltrating leukocytes and macrophages in pathological states [25]. iNOS generates higher concentrations of NO during a variety of inflammatory diseases independently of intracellular calcium concentration [26].
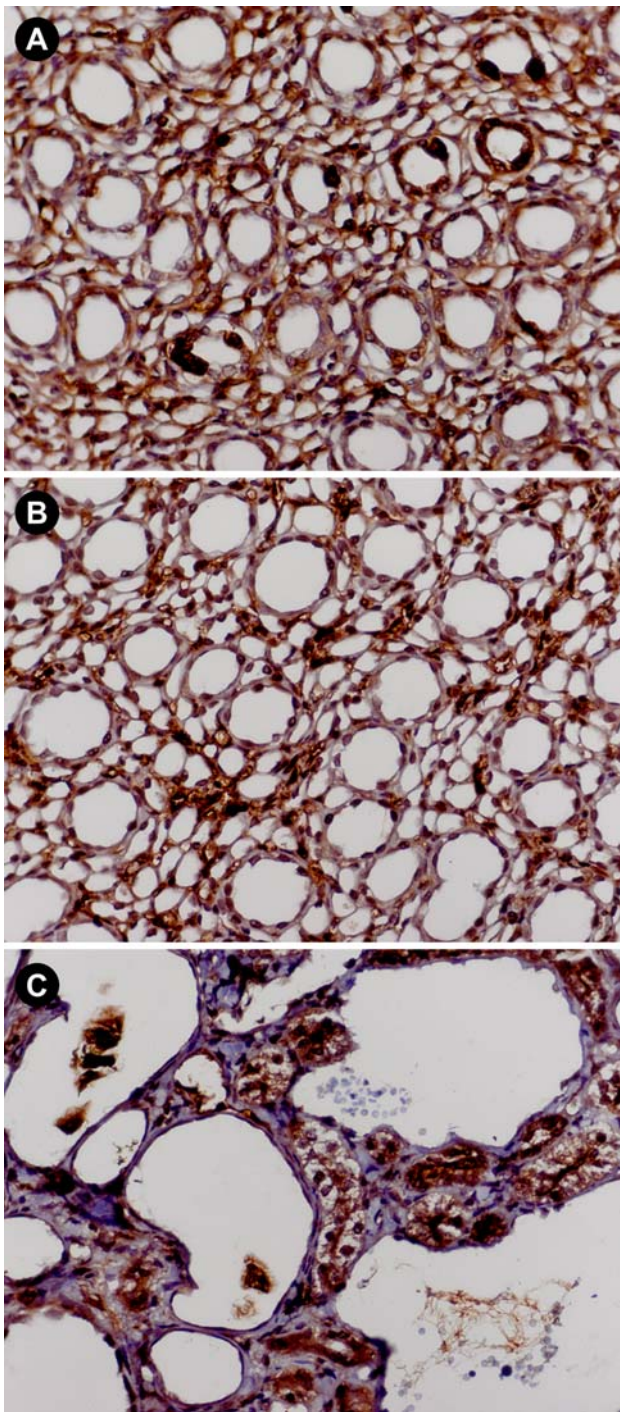
Fig. 3 Immunohistochemical analysis showing representative expression of inducible nitric oxide synthase (iNOS) at 14 days of ureteral obstruction. **a** Mild iNOS immunostaining is present in the control groups (1 and 3); **b** mild to moderate iNOS immunostaining is present in group 2; **c** marked iNOS immunostaining is present in group 4. Magnification, ×400

Fig. 4 Number of immunopositive cells for inducible nitric oxide synthase (iNOS) per renal tubule. iNOS expression in animals undergoing complete UO increased significantly compared with control sections at 14 days of obstruction. But this increase was more in group 4 when compared to group 2. $*p < 0.05$ vs. group 1, $**p < 0.05$ vs. group 3, $^{†}p < 0.05$ vs. group 2. Data are presented as mean ± SD

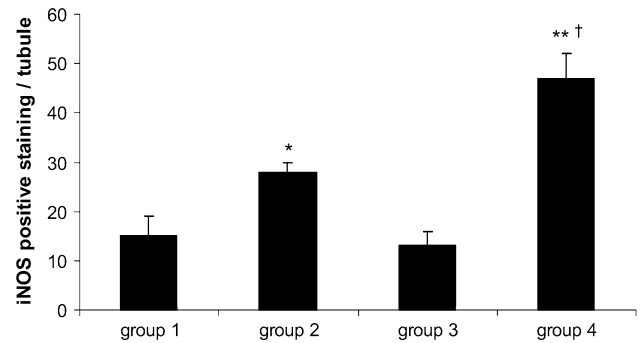Kipari et al. [5] showed that NO is the key mediator of macrophage-induced tubular epithelial cell apoptosis in vitro and plays a prominent role in vivo during tubulointerstitial inflammation. Also, they indicated that macrophage cytotoxicity in vitro is markedly dependent on iNOS-derived NO. These data are in accordance with our work indicating that inhibition of iNOS-derived NO production by propofol resulted in abrogation of tubular cell apoptosis compared to ketamine group, thereby suggesting that NO is the dominant mediator of tubular epithelial cell death induced by activated macrophages. Broadbelt et al. [20] also noted the early upregulation of iNOS expression and NO metabolites in pressurized renal epithelial cells. Similarly, elevated expression of iNOS was accompanied by a high NO production at 14 days of complete UO in our study. That eNOS immunoreactivity detected immunohistochemically was not increased as much as iNOS may suggests a reduction in the number of cells that express eNOS.

NO can directly act on the vasculature and cause systemic vasodilatation. However, excessive, unregulated NO production can also lead to the formation of reactive nitrogen species that can oxidize lipids, damage DNA, and cause protein modifications of important cellular proteins and enzymes [27]. The ability of NO or reactive nitrogen species to alter mitochondrial function and damage proteins may result in NO-mediated apoptosis or necrosis depending on the amount of NO and reactive nitrogen species generated [28].

As stated previously, apoptosis is the primary mechanism through which a reduction in renal mass occurs in response to obstruction, and in conjunction with interstitial fibrosis, eventually translates into irreversible renal dysfunction [29]. Choi et al. [30] demonstrated that the entire quantitative spectrum of tubular and interstitial cell apoptosis was maximal at 2 weeks of complete UO. Therefore, this time point was chosen in our work to study the effects of intravenous anesthetic agents on renal apoptosis and
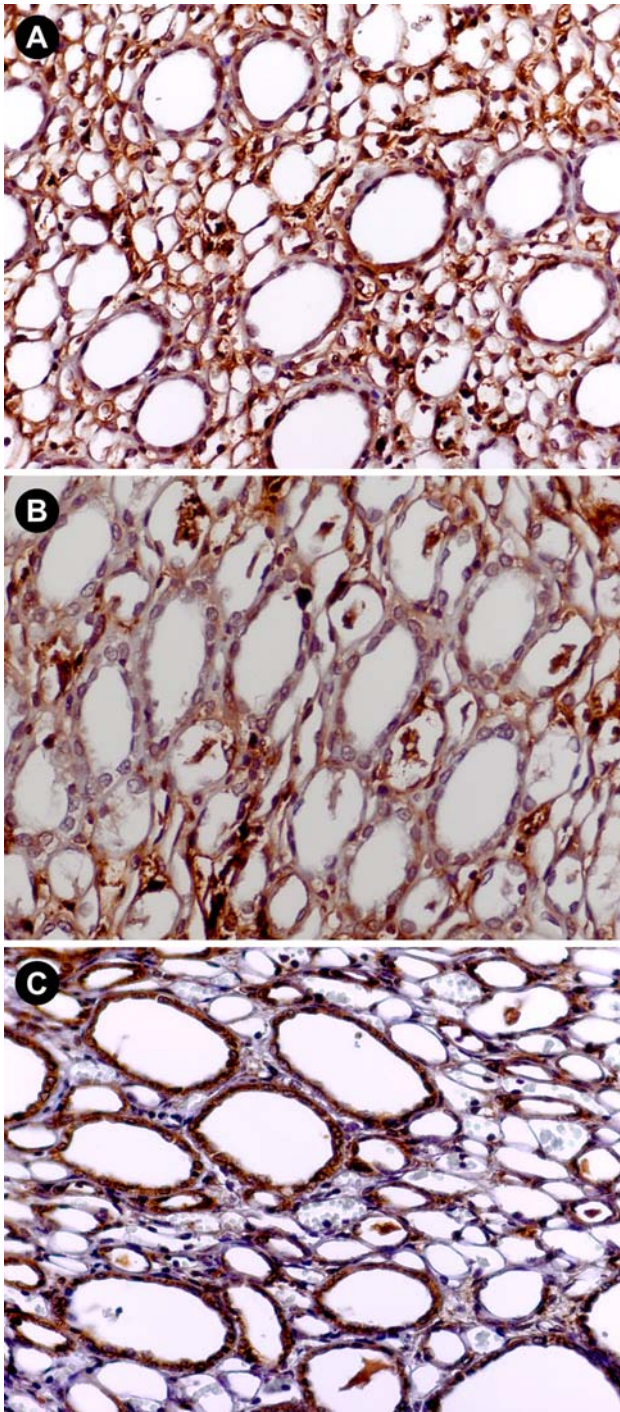
Fig. 5 Immunohistochemical analysis showing representative expression of apoptosis protease-activating factor 1 (APAF-1) at 14 days of ureteral obstruction. **a** Mild APAF-1 immunostaining is present in the control groups (1 and 3); **b** mild APAF-1 immunostaining is present in group 2; **c** marked APAF-1 immunostaining is present in group 4. Magnification, ×400

Fig. 6 Number of immunopositive cells for apoptosis protease-activating factor 1 (APAF-1) per renal tubule. Significantly increased numbers of apoptotic cells were detected in group 4, but only mild levels of apoptotic cells were present in rabbits undergoing complete UO under propofol anesthesia at 14 days after obstruction. *$p < 0.05$ vs. groups 3 and 2. Data are presented as mean ± SD

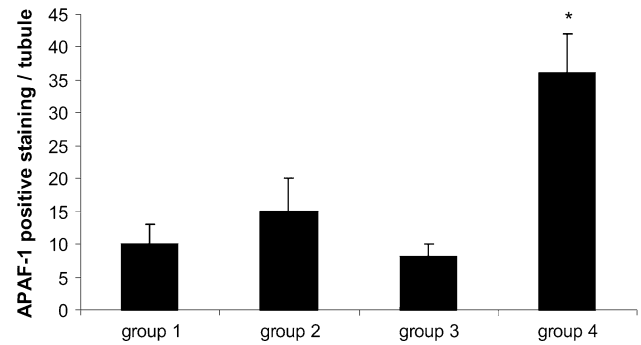eNOS and iNOS expressions. In the present study, propofol and ketamine were continuously infused for anesthesia of animals and preventive effects of these anesthetics were investigated and compared with each other at the same time. In this context, the present study is the first experimental study which investigated the effects of propofol and ketamine as anesthetics on both tissue eNOS and iNOS expressions and renal tubular cell apoptosis during complete UO. It is also of clinical importance to know the preventive effects of anesthetic agents on renal damage during UO.

APAF-1 is a 130 kDa protein that plays a central role in mitochondrial apoptosis. In response to apoptotic stimuli, such as an increase in NO levels and hypoxia, APAF-1 binds cytochrome c and procaspase-9 in the presence of adenosine triphosphate to form a multiproteic complex called the apoptosome [31]. The activation of procaspase-9 by autocatalytic cleavage initiates a cascade of downstream effector caspases, ultimately resulting in tubular cell apoptosis following UO [2].

Propofol has a chemical structure similar to that of phenol-based free-radical scavengers, such as butylated hydroxytoluene and the endogenous antioxidant α-tocopherol (vitamin E) [32]. Each molecule of propofol could scavenge two radical species and had significant antioxidant activity in vitro [9]. In our previous study, we found that propofol decreased NO-induced germ cell-specific apoptosis through downregulation of iNOS expression in an animal model of testicular torsion and detorsion [16]. Chen et al. [33] showed that propofol could inhibit the induction of iNOS and suppress the biosynthesis of NO in lipopolysaccharide-activated macrophages. Propofol is also a peroxynitrite scavenger and attenuates iNOS-mediated formation of peroxynitrite and apoptosis in cultured astrocytes [34]. The use of an antioxidant, such as α-tocopherol, in a tubulointerstitial disease, specifically unilateral UO, has previously been published [35, 36]. Decreased NO production and renal tubular cell apoptosis due to downregulation of iNOS expression by propofol in

our study seemed to be in line with these findings in the literature. Therefore, propofol is thought to decrease tubular cell apoptosis either by directly scavenging reactive oxygen species, or by decreasing iNOS-mediated formation of NO and peroxynitrite, or both. Even if an increase in the antioxidant capacity of tissue has been demonstrated during propofol anesthesia, blood propofol concentrations are not necessarily the most relevant in terms of the antioxidant properties of propofol [37]. Indeed, propofol is known to accumulate in biomembranes quite rapidly and may be able to boost the antioxidant defences of cells and tissues, especially in membranes [9, 12, 32]. Therefore, free-radical production, subsequent lipid peroxidation and iNOS expression and apoptosis after propofol anesthesia might have been prevented with propofol bound to proteins or present in membranes. The other possibility for the effects of propofol shown in the present study might be due to overdose of this drug administered for sacrifice of the animals. However, given the complexity of the action of propofol on iNOS expression and apoptosis in various tissues, this clearly is an area that warrants further exploration. Also, additional experiments using a different anesthetic agent for sacrifice of the animals can be an option in order to exclude the possible effects of propofol administered during sacrifice of the animals.

Ketamine is a rapid-acting general anesthetic which is indicated for a variety of diagnostic and short surgical procedures, to induce anesthesia prior to administration of other general anesthetics, and to supplement low-potency anesthetics such as nitrous oxide. Ketamine is often used for anesthesia in animals, during either veterinary procedures or for experimental purposes. The major adverse effects of ketamine include a variety of post-anesthesia symptoms, but this anesthetic does not elicit toxic reactions which are often associated with free radical formation, such as injury to the liver or kidney. Also, it is a suitable anesthetic for monitoring NO formation in the liver [38]. In this case, the NO radical is formed enzymatically by NOS, rather than indirectly through other oxidizing intermediates. Thus, we selected ketamine to compare the effect of propofol on oxidative stress. Our finding indicating that ketamine was not reduced iNOS-mediated NO production after complete UO is inconsistent with earlier studies reporting inhibitory effects of ketamine on the iNOS-mediated NO release from alveolar macrophages in response to lipopolysaccharide in vitro [39, 40]. The effect of ketamine on NO production from iNOS in vivo may thus be a result of additional mechanisms than those suggested by in vitro experiments, and further study in this area is merited.

Our data indicating that blockade of iNOS-derived NO by propofol anesthesia ameliorates the level of tubular cell apoptosis is not in accordance with previous work because NO has been implicated as a protective factor in the induction of tubular cell apoptosis by mechanical stretch in vitro and in vivo in ureteric obstruction [21]. The reasons for this are unclear, although experimental settings may be important because this can significantly affect the amount of NO generated by macrophages. It is undoubtedly the case that additional proapoptotic stimuli except the mechanical stretch, such as hypoxia, oxidative stress, cytokines, and so forth, also play a major role in the apoptosis of tubular epithelial cells [2].

Although our findings suggest that propofol is a promising anesthetic agent in case of complete UO induced kidney injury, our study had limitation. We did not have a negative control group in our study because of impossibility of performing this type of surgical intervention in rabbits without anesthesia.

In conclusion, the results from the current study indicate that complete UO via mechanisms involving increased iNOS expression and NO production could enhance renal tubular cell apoptosis. Propofol as an intravenous anesthetic may attenuate NO-induced renal tubular cell apoptosis through downregulation of iNOS expression in an animal model of complete UO. This protective effect of propofol may prove to be a promising approach in reducing damage in clinical situations.

References

1. Kin AC, Bohman SO. Renal structural and functional changes after unilateral ureteral obstruction in rabbits. Scand J Urol Nephrol. 1983;17:223–34.
2. Docherty NG, O'Sullivan OE, Healy DA, Fitzpatrick JM, Watson RW. Evidence that inhibition of tubular cell apoptosis protects against renal damage and development of fibrosis following ureteric obstruction. Am J Physiol Ren Physiol. 2006;290:F4–13.
3. Nguyen HT, Hsieh MH, Gaborro A, Tinloy B, Phillips C, Adam RM. JNK/SAPK and p38 SAPK-2 mediate mechanical stretch-induced apoptosis via caspase-3 and -9 in NRK-52 E renal epithelial cells. Nephron Exp Nephrol. 2006;102:e49–61.
4. Sunami R, Sugiyama H, Wang DH, Kobayashi M, Maeshima Y, Yamasaki Y, et al. Acatalasemia sensitizes renal tubular epithelial cells to apoptosis and exacerbates renal fibrosis after unilateral ureteral obstruction. Am J Physiol Ren Physiol. 2004;286:F1030–8.
5. Kipari T, Cailhier JF, Ferenbach D, Watson S, Houlberg K, Walbaum D, et al. Nitric oxide is an important mediator of renal tubular epithelial cell death in vitro and in murine experimental hydronephrosis. Am J Pathol. 2006;169:388–99.
6. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev. 1991;43:109–42.
7. Nathan C. Nitric oxide as a secretory product of mammalian cells. FASEB J. 1992;6:3051–64.
8. Blantz RC, Satriano J, Schwartz D. The renal response to sepsis and the role of NOS isoforms. Sepsis. 1998;1:131–4.
9. Murphy PG, Myers DS, Davies MJ, Webster NR, Jones JG. The antioxidant potential of propofol (2, 6-diisopropyl phenol). Br J Anaesth. 1992;68:613–8.

10. Runzer TD, Ansley DM, Godin DV, Chambers GK. Tissue antioxidant capacity during anesthesia: propofol enhances in vivo red cell and tissue antioxidant capacity in a rat model. Anesth Analg. 2002;94:89–93.

11. Cheng YJ, Wang YP, Chien CT, Chen CF. Small dose propofol sedation attenuates the formation of reactive oxygen species in tourniquet-induced ischemia-reperfusion injury under spinal anesthesia. Anesth Analg. 2002;94:1617–20.

12. Yagmurdur H, Cakan T, Bayrak A, Arslan M, Baltaci B, Inan N, et al. The effects of etomidate, thiopental, and propofol in induction on hypoperfusion-reperfusion phenomenon during laparoscopic cholecystectomy. Acta Anaesthesiol Scand. 2004; 48:772–7.

13. Weigand MA, Schmidt H, Zhao Q, Plaschke K, Martin E, Bardenheuer HJ. Ketamine modulates the stimulated adhesion molecule expression on human neutrophils in vitro. Anesth Analg. 2000;90:206–12.

14. Behrens MM, Ali SS, Dao DN, Lucero J, Shekhtman G, Quick KL, et al. Ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-oxidase. Science. 2007; 318:1645–7.

15. Yagmurdur H, Ayyildiz A, Karaguzel E, Ogus E, Surer H, Caydere M, et al. The preventive effects of thiopental and propofol on testicular ischemia-reperfusion injury. Acta Anaesthesiol Scand. 2006;50:1238–43.

16. Yagmurdur H, Ayyildiz A, Karaguzel E, Akgul T, Ustun H, Caydere M, et al. Propofol reduces nitric oxide-induced apoptosis in testicular ischemia-reperfusion injury by downregulating the expression of inducible nitric oxide synthase. Acta Anaesthesiol Scand. 2008;52:350–7.

17. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [^{15}N] nitrite in biological fluids. Anal Biochem. 1982;126:131–8.

18. Ricardo SD, Ding G, Eufemio M, Diamond JR. Antioxidant expression in experimental hydronephrosis: role of mechanical stretch and growth factors. Am J Physiol. 1997;272:F789–98.

19. Miyajima A, Chen J, Kirman I, Poppas DP, Darracott Vaughan EJR, Felsen D. Interaction of nitric oxide and transforming growth factor-β-1 induced by angiotensin II and mechanical stretch in rat renal tubular epithelial cells. J Urol. 2000;164:1729–34.

20. Broadbelt NV, Stahl PJ, Chen J, Mizrahi M, Lal A, Bozkurt A, et al. Early upregulation of iNOS mRNA expression and increase in NO metabolites in pressurized renal epithelial cells. Am J Physiol Ren Physiol. 2007;293:F1877–88.

21. Miyajima A, Chen J, Poppas DP, Darracott Vaughan EJR, Felsen D. Role of nitric oxide in renal tubular apoptosis of unilateral ureteral obstruction. Kidney Int. 2001;59:1290–303.

22. Blaylock MG, Cuthbertson BH, Galley HF, Ferguson NR, Webster NR. The effect of nitric oxide and peroxynitrite on apoptosis in human polymorphonuclear leukocytes. Free Radic Biol Med. 1998;25:748–52.

23. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch Biochem Biophys. 1991; 288:481–7.

24. Moulian N, Truffault F, Gaudry-Talarmain YM, Serraf A, Berrih-Aknin S. In vivo and in vitro apoptosis of human thymocytes are associated with nitrotyrosine formation. Blood. 2001;97:3521–30.

25. Kone BC. Nitric oxide in renal health and disease. Am J Dis. 1997;30:311–33.

26. Aktan F. iNOS-mediated nitric oxide production and its regulation. Life Sci. 2004;75:639–53.

27. Dedon PC, Tannenbaum SR. Reactive nitrogen species in the chemical biology of inflammation. Arch Biochem Biophys. 2004;423:12–22.

28. Meij JT, Haselton CL, Hillman KL, Muralikrishnan D, Ebadi M, Yu L. Differential mechanisms of nitric oxide- and peroxynitrite-induced cell death. Mol Pharmacol. 2004;66:1043–53.

29. Chevalier RL. Pathogenesis of renal injury in obstructive uropathy. Curr Opin Pediatr. 2006;18:153–60.

30. Choi JY, Baranowska-Daca E, Nguyen V, Koji T, Ballantyne CM, Sheikh-Hamad D, et al. Mechanism of chronic obstructive uropathy: increased expression of apoptosis-promoting molecules. Kidney Int. 2000;58:1481–91.

31. Ashraf QM, Mishra OP, Delivoria-Papadopoulos M. Mechanisms of expression of apoptotic protease activating factor-1 (APAF-1) in nuclear, mitochondrial and cytosolic fractions of the cerebral cortex of newborn piglets. Neurosci Lett. 2007;415:253–8.

32. Demiryurek AT, Cinel I, Kahraman S, Tecder-Unal M, Gogus N, Aypar U, et al. Propofol and intralipid interact with reactive oxygen species: a chemiluminescence study. Br J Anaesth. 1998; 80:649–54.

33. Chen RM, Wu GJ, Tai YT, Sun WZ, Lin YL, Jean WC, et al. Propofol reduces nitric oxide biosynthesis in lipopolysaccharide-activated macrophages by downregulating the expression of inducible nitric oxide synthase. Arch Toxicol. 2003;77:418–23.

34. Acquaviva R, Campisi A, Murabito P, Raciti G, Avola R, Mangiameli S, et al. Propofol attenuates peroxynitrite-mediated DNA damage and apoptosis in cultured astrocytes: an alternative protective mechanism. Anesthesiology. 2004;101:1363–71.

35. Saborio P, Krieg RJ, Kuemmerle NB, Norkus EP, Schwartz CC, Chan JCM. α-Tocopherol modulates lipoprotein cytotoxicity in obstructive nephropathy. Pediatr Nephrol. 2000;14:740–6.

36. Chan W, Krieg RJ, Ward K, Santos F, Lin KC, Chan JCM. Progression after release of obstructive nephropathy. Pediatr Nephrol. 2001;16:238–44.

37. Hans P, Deby-Dupont G, Doby C, Pieron F, Verbesselt R, Franssen C, et al. Increase in antioxidant capacity of plasma during propofol anesthesia. J Neurosurg Anesthesiol. 1997; 9:234–6.

38. Reinke LA, Moore DR, Kotake Y. Hepatic nitric oxide formation: spin trapping detection in biliary efflux. Anal Biochem. 1996;243:8–14.

39. Li CY, Chou TC, Wong CS, Ho ST, Wu CC, Yen MH, et al. Ketamine inhibits nitric oxide synthase in lipopolysaccharide-treated rat alveolar macrophages. Can J Anaesth. 1997; 44:989–95.

40. Shibakawa YS, Sasaki YS, Goshima Y, Echigo N, Kamiya Y, Kurahashi K, et al. Effects of ketamine and propofol on inflammatory responses of primary glial cell cultures stimulated with lipopolysaccharide. Br J Anaesth. 2005;95:803–10.