ORIGINAL ARTICLE

The renal protective efficacy of ketamine vs. propofol vs. ketofol against renal ischemia / reperfusion injury: An experimental rat study

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Abstract

The purpose of this study was to investigate the renal protective efficacy of propofol, ketamine, and ketofol in an ischemia/reperfusion injury (IRI) model experimentally induced in rats. Thirty-five male Sprague-Dawley rats were randomly assigned into five groups: sham (Group 1), control (Group 2), ketamine (Group 3), propofol (Group 4), and ketofol (Group 5). Following administration of intramuscular ketamine hydrochloride anesthesia, the left renal hilum was exposed to 60-minutes clamping. Ketamine (Group 3), propofol (Group 4), and ketofol (Group 5) were administered 15 min prior to reperfusion. After six hours of reperfusion, the left kidneys were harvested for histological injury scoring. Mean histopathology scores were 0.42±0.53 in group 1 (Sham), 2.97±0.21 in group 2 (Control), 2.68±0.15 in group 3 (Ketamine), 2.40±0.16 in group 4 (Propofol), and 2.51±0.22 in group 5 (Ketofol). The score in group 4 (Propofol) was significantly lower than those in groups 2 (control) and 3 (Ketamine) (P=.002 and P=.0120, respectively), but did not differ significantly from that in group 5 (Ketofol) (P=.347). The score in group 5 (Ketofol) was significantly lower compared to group 2 (control) (P=.006), but no significant difference was determined with Group 3 (Ketamine) (P=.137). No significant difference was also observed between Group 3 (Ketamine) and Group 2 (control) (P=.021). The study results indicated no superiority of ketofol over ketamine or propofol against renal IRI. However, the renal protective effect of propofol against IRI was greater than that of ketamine.

Keywords: Propofol, ketofol, ketamine, ischemia reperfusion injury, kidney

Introduction

Ischemia reperfusion injury (IRI) represents cellular damage occurring following reperfusion of formerly viable ischemic tissues [1]. Inflammation plays a substantial role in the pathogenesis of IRI, with central involvement for particular cells, adhesion molecules and cytokines. Hypoxia caused by ischemia and subsequent reperfusion is linked to an increase in the production of reactive oxygen species (ROS), which are generated on an extensive basis during IRI and which mediate cellular damage. Oxidative stress is the result of disequilibrium between antioxidant capacity and ROS production. Important factors by which renal IRI leads to tubular cell injury and death include the formation of ROS and oxidants/ antioxidant imbalance [2]. Renal ischemia and reperfusion injury may be caused by systemic hypotension, cardiac arrest, hypovolemic shock, major trauma, tissue injury, renovascular surgery, and aortic clamping. Acute kidney injury developing secondary to IRI plays a major role in perioperative morbidity and mortality, and constitutes a significant health care and economic burden [3,4]. Acute kidney injury occurs in approximately 1% of surgical cases [5].

Propofol is a gamma-aminobutyric acid A receptor that enhances its inhibitory activity and is the intravenous anesthetic agent most widely employed for the purpose of inducing and maintaining general anesthesia. Propofol has also been evaluated in terms of its renoprotective effects in IRI and other organs. The specific molecular mechanism involved in the protective effect exhibited by propofol in the face of renal IRI is unknown, although it exhibits protective activities through antioxidant and anti-inflammatory effects and the downregulation of proinflammatory cytokine production. Propofol upregulates heme oxygenase-1, which in turn increases carbon monoxide (CO), free ferrous iron,

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and biliverdin levels. Free ferrous iron and biliverdin and exhibit antioxidant effects by scavenging environmental free radicals. CO is a signaling molecule, the anti-inflammatory effects of which involve the downregulation of proinflammatory cytokines such interleukin-1b (IL-1b) and tumor necrosis factor-a (TNF-a) as well as the upregulation of anti-inflammatory cytokines via mitogen-activated protein kinase kinase-3 (MAPKK-3). In addition, propofol exhibits protective effects in renal tissue by activating adenosine triphosphate-sensitive potassium channels and significantly reduces IRI-associated apoptosis in kidney cells. Propofol also contains a phenol hydroxyl group in its chemical structure. Phenol hydroxyl reacts with peroxynitrite, a highly reactive oxidant and a product of lipid metabolism, to remove it from the environment [2]. Ketamine is an N-methyl-D-aspartate antagonist and dissociative anesthetic agent that lowers IRI. Research has demonstrated that it exhibits a protective effect against IRI, although the exact molecular mechanisms involved are still unclear [6-9]. Ketamine has been shown to reduce the generation of pro-inflammatory cytokines, to attenuate induced lipid peroxidation, and also to enhance renal antioxidant status, thus exhibiting a protective effect against renal IRI [6,8]. Ketofol consists of a combination of propofol 0.5mg kg^{-1} and ketamine 0.5mg kg^{-1}. It has been shown to improve recovery time, and there is also evidence that it lowers the frequency of single agents involved in hypotension [10]. The combined use of ketamine and propofol can alter their renal protective effects against IRI. The present study involved a histopathological investigation of the synergistic effect of a ketamine+propofol combination on the management of renal IRI.

Material and Methods

Animals

The experimental protocol adopted in the current study was approved by the Animal Ethics Review Committee of the Gaziosmanpasa University, Turkey (no. 51879863-186, Date: 09.12.2021). Thirty-five male Sprague-Dawley rats weighing 341.35±11.05 g (range, 321-363 g) and aged 9-12 weeks were obtained from the Gaziosmanpasa Experimental Animals Laboratory (Tokat, Turkey). These were housed in individual cages within a specific-pathogen-free environment at 22±1°C, at a relative humidity of 40-70%, and in a 12h/12h light/dark cycle. Ad libitum access was permitted to food and water. The rats were cared for as specified by the guidelines related to the care and use of laboratory animals issued by the U.S. National institutes of Health (NIH Publication No. 85-23, revised 2011) [11]. This study was also conducted in conformity with the ARRIVE animal experiments guideline [12].

Ischemia reperfusion injury model

All rats were fasted for 12h before being anesthetized using intramuscular injection of 60 mg kg^{-1} ketamine hydrochloride. The abdomen was first shaved and sterilized with povidone iodine solution, after which a 3-cm midline incision was made, and the abdominal viscera were displaced to the right. The left renal hilus was then dissected, after which the renal vascular pedicle was occluded with the attachment of a microvascular clamp. The ketamine dose was repeated 30 minutes later intramuscularly. The clamps were released after 60 minutes of ischemia. The

bloodstream in the renal artery and vein was observed, as indicated by the restoration of its original color, after which the abdomen was closed, and the rats were woken. Following six-hour reperfusion, the rats were sedated using intramuscular injection of 60mg kg^{-1} ketamine hydrochloride. Left nephrectomy was then performed, after which all experimental animals were euthanized with a lethal intraperitoneal (IP) dose of ketamine.

Experimental design

Following a seven-day adaptation period, the 35 male rats were randomly assigned into sham, control, and three experimental groups in equal numbers (n=7) as follows:

Group 1 (Sham): Laparotomy was performed following anesthesia in this group. Left nephrectomy was performed six hours after laparotomy. No IRI model was employed.

Group 2 (Control): An IRI model was applied to the rats in this group. Left nephrectomy was performed six hours after laparotomy. No IRI agent was used.

Group 3 (Ketamine): An IRI model was applied to the rats in this group. Fifteen minutes before reperfusion, 10mg kg^{-1} ketamine (Ketalar, Eczacibasi, Turkey) was administered via the IP route. Left nephrectomy was performed six hours after laparotomy.

Group 4 (Propofol): An IRI model was applied to the rats in this group. Fifteen minutes before reperfusion, 10mg kg^{-1} propofol (Propofol-PM 1%, Polifarma, Turkey) was administered IP. Left nephrectomy was performed six hours after laparotomy.

Group 5 (Ketofol): An IRI model was applied to the rats in this group. Fifteen minutes before reperfusion, 10mg kg^{-1} ketofol was administered IP. Left nephrectomy was performed six hours after laparotomy (Figure 1).
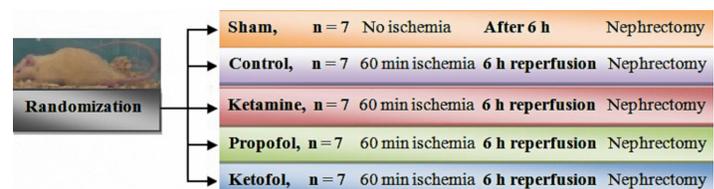

Figure 1. The rats were randomized into five different groups

Histopathology and scoring of renal injury

The extracted kidneys were fixed in formalin, following which sections taken from the upper, middle, and lower parts were embedded in paraffin. Multiple three-micrometer sections were cut and stained with hematoxylin/eosin (H&E). During quantitative analysis of histological patterns, tissue changes observed after I/R were scored from 0 to 5 (0=normal kidney, 0% injury; 1= minimal damage, <10% injury; 2=mild damage, 11-25% injury;3 =moderate damage, 26-45% injury; 4=severe damage, 46-75 injury; and 5=very severe damage, >76% injury). The scoring was based on changes in intracellular edema, atypical cytoplasmic vacuolization, cell detachment, tubular dilatation, and tubular necrosis (Figure 2). Histological kidney injury was assessed by an experienced uropathologist (MB) blinded to the study groups. A minimum of 10 high-power fields (HPFs; magnification, x200) were examined per section for each sample [13].
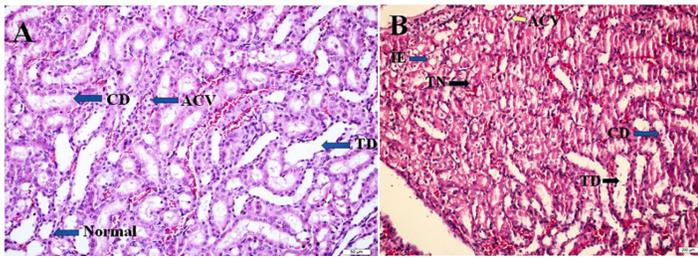
Figure 2. Histology after one hour of ischemia and six hours of reperfusion demonstrating the different morphological changes scored by the pathologist with Hematoxylin-Eosin staining. A (left); mild kidney damage (x400). B (right); severe kidney damage (x200). ACV: Atypical cytoplasmic vacuolization; CD: Cell detachment; IE: Intracellular edema; TD: Tubular dilatation; TN: Tubular necrosis

Statistical analysis

All statistical analyses were carried out on computerized software (IBM SPSS version 25, Chicago, IL, USA). Data are presented as mean±standard deviation. A significance level of 0.05 was employed. Differences were analyzed by the application of one-way ANOVA and the H-test. The Bonferroni-corrected Mann-Whitney U test was employed to identify the source of significance in variables determined to be as significant.

Results

Mean weights were 341.28±10.91 g (range, 322–357 g) in Group 1, 340.28±10.35 g (range, 323–357 g) in Group 2, 340.57±11.53g (range, 323–358 g) in Group 3, 340.85±13.24 g (range, 321–358 g) in Group 4, and 342.71±10.91 g (range, 329–363 g) in Group 5 (P=.993). In Group 1 (Sham), the scores for all the histological values analyzed were quite low, significantly lower than in the ischemia groups. The highest histological value in Group 1 (Sham) was 0.57±0.53 for intracellular edema, while the lowest score, 0, was determined for tubular necrosis.

In Group 2 (Control), all histological scores-atypical cytoplasm vacuolization, cell detachment, intracellular edema, tubular dilatation, and tubular necrosis were higher than in the other groups. However, this elevation was found to be only statistically significant for tubular necrosis. The lowest tubular necrosis score was observed in Group 4 (Propofol), significantly lower compared to Group 2 (Control). The comparable scores in Group 3 (Ketamine) and Group 5 (Ketofol) were not significant compared to Group 2 (Control).

Mild atypical cytoplasm vacuolization and cell detachment were observed in the three study groups – Group 3 (Ketamine), Group 4 (Propofol), and Group 5 (Ketofol). The scores were similar in all three groups, with no significant difference between them (P=.513 and P=.517, respectively). The most pronounced, albeit moderate, intracellular edema and tubular dilatation were observed in Group 3 (Ketamine). Scores in Group 4 (Propofol) and Group 5 (Ketofol) were lower than those in Group 3 (Ketamine), but the differences were not significant (P=.179 and P=.260, respectively). No tubular necrosis was observed in Group 1 (Sham), although mild necrosis was observed in the other groups. Tubular necrosis scores were higher in Group 3 (Ketamine) than in Group 4 (Propofol) and Group 5 (Ketofol), but this was not significant (P=.562).

Mean histological scores were 2.97±0.21 in Group 2 (Control), 2.68±0.15 in Group 3 (Ketamine), 2.40±0.16 in Group 4 (Propofol), and 2.51±0.22 in Group 5 (Ketofol) (Figure 3). This low value in Group 3 (Propofol) was significant compared to Group 2 (Control) and Group 3 (Ketamine) (P=.002 and P=.0120, respectively), but not compared to Group 5 (Ketofol) (P=.347). The score in Group 5 (Ketofol) was significantly lower than in Group 2 (Control) (P=.006), while no significant difference was determined compared to Group 3 (Ketamine) (P=.137). No difference was also determined between Group 3 (Ketamine) and Group 2 (Control) (P=.021). The groups' histological scores are shown in Table 1.
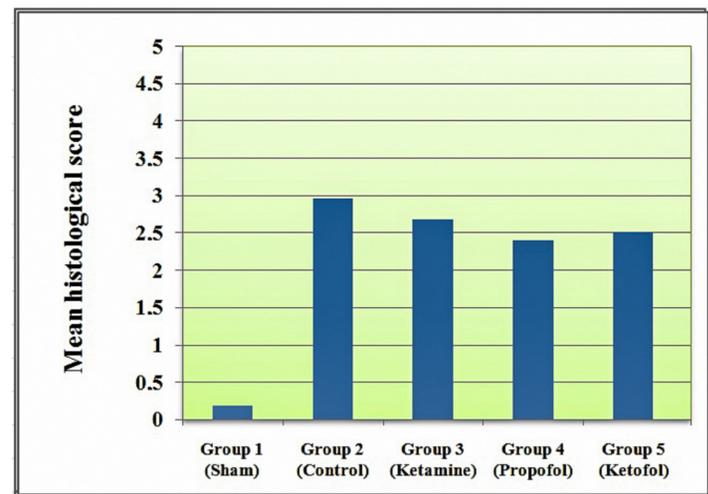

Figure 3. Mean histological scores in the study groups

Table 1. Histology score in the groups

Histology	Group 1 (Sham)	Group 2 (Control)	Group 3 (Ketamine)	Group 4 (Propofol)	Group 5 (Ketofol)
Atypical cytoplasm vacuolization	0.42±0.53*	2.85±0.69	2.42±0.53	2.14±0.37	2.28±0.48
Cell detachment	0.28±0.48*	3±0.57	2.85±0.37	2.57±0.78	2.85±0.69
Intracellular edema	0.57±0.53*	3.14±0.69	3.14±0.37	2.71±0.48	2.85±0.37
Tubular dilatation	0.14±0.37*	3±0.57	2.85±0.37	2.42±0.53	2.57±0.53
Tubular necrosis	0*	2.71±0.48	2.28±0.48	2±0.57**	2.14±0.37
Mean score	0.18±0.06*	2.97±0.21	2.68±0.15	2.40±0.16#,Δ	2.51±0.22##

*Statistically significant difference (P<.05) according to ischaemic groups, **Significantly different from the Control group (P=.0124), #Significantly different from the Control group (P=.002), ##Significantly different from the Control group (P=.006), ΔSignificantly different from the Ketamine group (P=.012)

Discussion

Acute kidney injury developing secondary to IRI is a significant factor in preoperative morbidity and mortality, and constitutes a considerable healthcare and financial burden. It also results in new onset of chronic kidney disease, as well as accelerating its course [2]. Numerous different agents have to date been used to reduce renal IRI, including anesthetics [2,14]. Propofol and ketamine have been shown to exhibit a renal protective effect against IRI in previous experimental rat models. However, we are aware of no previous investigation of the effect on renal IRI of ketofol, a mixture of equal quantities of propofol and ketamine. The present research was therefore intended to examine the renal protective effect of ketofol against IRI, and observed this to be similar to those of propofol and ketamine [14].

Although the mean histological score value for propofol was lower than that of ketofol, the difference was not significant, with both agents exhibiting similar renal protective properties against IRI. In their experimental rat studies investigating the renal protective effect of propofol against renal IRI, Yuzbasioglu et al. and Li et al. observed a greater protective effect compared to control groups, and attributed this to the antioxidant properties of propofol [15,16].

The principal finding in the current research was the significantly lower mean histological score in the propofol group compared to the ketamine group, suggesting the presence of a renal protective effect of propofol in this model. Similarly to the present study, Yuzer et al. investigated the renal protective effects against renal IRI of intravenous anesthetics in their experimental rat study. Those authors reported lower histological scores for propofol compared to ketamine, although the difference was not statistically significant [9]. In contrast to Yuzer et al., the difference between the two substances in the present study was statistically significant. In their IRI study, involving the same design as the present research, Dogan et al. subjected rats to biochemical and serological investigation and observed higher malondialdehyde levels and lower catalase activities and superoxide dismutase levels in the propofol group compared to the ketamine group [17]. Similarly to the present study, propofol was found to provide more effective protection against renal IRI than ketamine.

No significant difference was determined in mean histological score values between the control group and the ketamine group in this study. Ketamine was employed at a dosage of 10mg kg^{-1} in the present research. In their experimental rat study investigating the dosage at which ketamine is most effective against renal IRI, Demirkiran et al. employed doses of 3, 10, 30, 60, and 80mg kg^{-1} and found that a dosage of 3mg kg^{-1} significantly reduced IRI in rats. Those authors recommended that ketamine be used at 3mg kg^{-1} against IRI [7]. We think that the absence of any difference between the ketamine and control groups in the present study may be due to our use of ketamine at a dosage with a lower antioxidant effect, 10mg kg^{-1}.

Intracellular edema, atypical cytoplasmic vacuolization, cell detachment, tubular dilatation, and tubular necrosis were subjected to separate histopathological evaluations and were assigned numerical values. All these subscores were lower in the propofol group than in the other ischemia groups. The control group subscores were higher than in the other ischemia groups. However, this variation was not statistically significant.

For technical reasons, antioxidant agents such as superoxide dismutase, glutathione peroxidase, and malondialdehyde in serum or tissues, proinflammatory cytokines such as TNF-a, IL-1b, IL-6, serum urea, and creatinine for the purpose of evaluating kidney functions, and urinary albumin and urinary albumin/creatinine ratio levels to indicate acute renal injury could not be measured in this study. This represents the principal limitation of this research. However, despite these deficiencies and the fact that only histopathological examination could be performed, we think that this study is still particularly valuable as one of the first to investigate the renal protective effect of ketofol against renal IRI.

Conclusion

The primary objective of the current research was to examine the protective effect of ketofol against IRI in kidney tissue compared to ketamine and propofol. Ketofol was found to exhibit no superiority over either ketamine or propofol. However, propofol exhibited a greater protective effect against IRI in kidney tissue than ketamine. We conclude that propofol is a more appropriate choice for the induction and maintenance of anesthesia in surgical procedures such as partial nephrectomy or renal transplantation in which IRI will occur.

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