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Original Article

A single small dose of ketamine prevents lung injury following hepatic ischemia-reperfusion in rabbits

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Abstract

Background: Previous studies demonstrated that large-dose ketamine alleviated lipopolysaccharide-induced lung injury. We investigated whether a single small dose of ketamine could attenuate lung injury induced by hepatic ischemia-reperfusion (HIR) and, if so its underlying mechanisms.
Methods: Thirty male New Zealand rabbits were randomized into three groups ($n = 10$ each): sham group (Group S), control group (Group C), and ketamine group (Group K). In Group S, hepatic portal vein (HPV) and inferior vena cava (IVC) were left unclamped and 0.9% saline 1 mL/kg was given intravenously. In Group K, ketamine 0.5 mg/kg (0.5 mg/mL) was given intravenously 10 minutes before cross-clamping of the HPV and the IVC. In Group C, 0.9% saline was given 10 minutes before the cross-clamping. The HPV and the IVC were cross-clamped with bulldog clamps and unclamped 60 minutes later in the Group K and the Group C. Blood pressure and pulse rate were recorded throughout the procedure. Rabbits were sacrificed 6 hours postoperatively. Lung W/D ratio was calculated and expression of tumor necrosis factor-α mRNA, intracellular adhesion molecule-1 mRNA, and nuclear factor kappa B (NF-κB)/p65 were quantitatively analyzed. Accumulation of neutrophils in lung tissues was also observed.
Results: Small dose of ketamine alleviated the pulmonary edema, but not the systemic hypotension, induced by cross-clamping of the IVC and the HPV. Pretreatment with ketamine significantly reduced the increments of tumor necrosis factor-α mRNA, intracellular adhesion molecule-1 mRNA, and NF-κB/p65; and inhibited the aggregation of neutrophils in lung tissues following HIR.
Conclusion: 0.5 mg/kg ketamine pretreatment showed significant protective effect on acute lung injury induced by HIR, which might be mediated by the NF-κB pathway.

Keywords: Hepatic ischemia-reperfusion; Ketamine; Lung injury; Proinflammatory cytokines

1. Introduction

Major liver surgeries such as orthotopic liver transplantation (OLT) often require cross-clamping of the inferior vena cava (IVC) and hepatic portal vein (HPV) to reduce excessive blood loss and improve surgical condition. Release of the clamps from the IVC and HPV after long duration of cross-clamping often leads to serious hypotension, which may induce acute lung injury.[1,2] One of the major mechanisms underlying acute lung injury is accumulation of proinflammatory cytokines such as tumor necrosis factor (TNF)-α and intracellular adhesion molecule (ICMA)-1 in the lung, which induce pulmonary aggregation of activated lymphocytes and neutrophils, provoking aggressive inflammatory response.[3] The nuclear factor kappa B (NF-κB) pathway plays an important role in mediating and modulating proinflammatory cytokines.[4–6]

Previous clinical studies proved that ketamine, an intravenous anesthetic, could inhibit elevation of proinflammatory cytokines,[7] which may have protective effect on acute lung injury. Ketamine suppresses proinflammatory cytokine production *in vitro* and dose-dependently inhibits increase of TNF-α and interleukin-6 in lipopolysaccharide-induced acute lung injury in rats.[8,9] However, ketamine sometimes causes dramatic hemodynamic changes, which may induce serious pulmonary artery hypertension, provoking or aggravating pulmonary edema.[10]

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In this study, a single small dose of ketamine was given to rabbits before cross-clamping of the IVC and the HPV, changes in systematic hemodynamics and pulmonary proinflammatory cytokines after hepatic reperfusion were observed to evaluate the effect of ketamine preconditioning on hepatic ischemia-reperfusion (HIR) induced acute lung injury.

2. Methods

2.1. Animal model

Thirty male New Zealand rabbits, weighed 2–2.5 kg (purchased from Animal Centre of Shanghai First People's Hospital affiliated to Shanghai Jiaotong University) were randomized into sham group [(Group S) $n = 10$], control group [(Group C), $n = 10$], and ketamine group [(Group K), $n = 10$]. All animals were housed in standard conditions (temperature 25°C with a 12 hours light and 12 hours darkness) and allowed free access to tap water and food. All experimental animal protocols were performed in accordance with guidelines approved by the Animal Care and Use Committee at Shanghai First People's Hospital affiliated with Shanghai Jiaotong University.

A 24G catheter was inserted in the rabbit ear median artery and connected to a pressure transducer P-260 (Edward Lifescience Co., Irvine, CA, USA) for arterial blood pressure measurement. 0.9% saline (Baxter Company, Deerfield, IL, USA) was infused at 15 mL/kg/h by means of a 24G catheter in the ear vein by a TOP syringe pump (Top Company, Tokyo, Japan). Rabbits were anesthetized with 3% pentobartital 30 mg/kg intravenously. The HPV and the IVC were exposed after laparotomy. In Group S, the HPV and the IVC were left unclamped and 0.9% saline 1 mL/kg was given intravenously. In Group K, ketamine 0.5 mg/kg (0.5 mg/mL) was given intravenously 10 minutes before cross-clamping of the HPV and the IVC. In Group C, 0.9% saline was given 10 minutes before the cross-clamping. The HPV and the IVC were cross-clamped with bulldog clamps and unclamped 60 minutes later in Group K and Group C. The abdominal incision was then sutured and all rabbits were sacrificed 6 hours after the operation.

2.2. Tissue specimens

After the sacrifice, the lungs were taken out. The right lung was used to measure the dry-weight to wet-weight ratio (W/D ratio). Part of the left lung was preserved in 10% formaldehyde for later immunohistochemical stain. The rest of the left lung was cryopreserved in liquid nitrogen for further Western blot analysis and real-time quantitative polymerase chain reaction (RT-PCR) tests.

2.3. Measurement of lung W/D ratio

The right lung was blotted after being rinsed with 0.9% saline. It was weighed and then dried at 110°C for 24 hours. The dried tissue was again weighed and the W/D ratio was calculated.

2.4. Immunohistochemical stain

Quantity of ICMA-1 and accumulation of neutrophils in the lung tissue was analyzed by immunohistochemical enzyme-linked immunosorbent assay stain. The lung tissue was fixed in 10% formaldehyde and sliced into 4–6 μm sections after paraffin imbedding. The sections were then dyed with hematoxylin and eosin (HE) stain and treated with 3% hydrogen dioxide solution to block the edogenous peroxidase and biotin activities. After incubation with goat-anti-rabbit ICAM-1 antibody at 4°C for 16 hours, biotinylated anti-goat IgG, streptavidin-peroxidase, and 3,3′-diaminobenzidine were used for immunohistochemical stain. Goat serum was used as wash and buffer solution throughout the whole procedure. Sections were counterstained with HE. Three different areas in each HE section were analyzed within a 100 μm scope.

2.5. Mean artery pressure and pulse rate monitoring

Mean artery pressure (MAP) was continuously monitored and the pulse rate (PR) was collected by the count of arterial waveform. The MAP and the PR were recorded at 10 minutes after the exposure of the HPV and the IVC without clamping (t1), 10 minutes after cross-clamping of the HPV and the IVC (t2) and 10 minutes after unclamping (t3).

2.6. Real-time quantitative polymerase chain reaction analysis

Expression of mRNA encoding TNF-α and ICAM-1 in frozen lung tissue were quantitatively analyzed by the RT-PCR with TRIZOL kit (Invitrogen company, Carlsbad, CA, USA) according to the manufacturer's instructions. Total volume of reverse transcription was 20 μL and total content of tissue was 2 μg. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as reference, and the number of RT-PCR copies was 30.

Below are the sequences of primers for the PCR amplification:

GAPDH: sense:5′-TGATCCATTCATTGACCTCC-3′ (307 bp); antisense:5′-GTTCACGCCCATCACAAACA-3′ (307 bp); TNF-α: sense:5′-TAGTAGCAAACCCGCAAGT -3′ (421 bp); antisense:5′-AGGGCAATGATCCCAAAGT-3′ (421 bp).

ICAM-1: sense:5′-TCTCCTTGGGGTCCCCATCC-3′(194 bp) antisense:5′-TGCCACCATCACTGTGTATT-3′ (194 bp).

The buffered RT-PCR products and the GAPDH were electrophoresed in 1.8% agarose gel (Invitrogen company, Carlsbad, CA, USA) at 80 V for 1 hour. The gel was stained with 0.5 mg/mL ethidium bromide in Tris/borate/EDTA buffer. Quantit One 4.3.1 software (Bio-rad Laboratories, Atlanta, GA, USA) was used for analysis.

2.7. Western blot analysis

NF-κB/p65 in lung tissue was detected by Western blot analysis. Lung tissue homogenate was centrifuged, and the supernatant (50 μg protein) was electrophoresed in 10%

sodium dodecyl sulfate-polyacrylamide gel at 150 V for 1 hour. The protein was then electroblotted to a 0.45-μm nitrocellulose membrane (Millipore Co, Kankakee, IL, USA). The membrane was blocked for 1 hour at room temperature with blocking solution (5% nonfat milk in Tris-buffered saline with Tween 20). Incubated overnight at 4°C with rabbit polyclonal anti-NF-κB/p65 antibody (1:500 dilution, Beijing Boisynthesis Biotechnology Co, Ltd, Beijing, China), the blots were washed three times and incubated with a horseradish peroxidase-labeled secondary antibody for 1 hour at room temperature. Immunoreactive proteins were visualized with the use of enhanced chemiluminescence detection (Thermo Fisher Scientific Co, Rockford, IL USA). The images were processed with Quantit One 4.3.1 (Bio-rad) software.

2.8. Statistical analysis

Data were expressed as mean ± standard deviation. Tukey's *post hoc* test two-tailed analysis of F test in one way analysis of variance was applied for comparison between groups and within groups. Correlation between TNF-α, ICAM-1 mRNA, and NF-κB/p65 was analyzed by Pearson's correlation analysis. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Lung W/D ratio

Group S showed the lowest W/D ratio among all the groups. W/D ratio was significantly lower in Group K than in Group C ($p < 0.05$) (Table 1).

3.2. Lung histology and immunohistochemical stain

Group S showed normal pulmonary histology. In contrast, the lung tissues from Group C and Group K were significantly damaged, with interstitial edema, hemorrhage, thickening of the alveolar wall and infiltration of inflammatory cells in interstitium and alveoli. These histological changes were less pronounced in Group K (Fig. 1).

There was no detectable ICAM-1 in the pulmonary interstitium in Group S. ICAM-1 and polymorphonuclear neutrophil (PMN) accumulation could be found in both Group C and Group K, whereas Group C showed higher density and intensity of ICAM-1 staining, as well as more PMN accumulation than Group K (Fig. 1).

3.3. MAP and PR

There was no significant difference in the MAP and the PR among all groups at t1 (MAP F = 1.479, $p = 0.267$, PR F = 1.209, $p = 0.424$). The MAP and the PR remained stable in Group S during the whole procedure (MAP F = 0.544, $p = 0.878$, PR F = 1.252, $p = 0.390$). In Group C, the MAP reduced significantly after the clamping of the HPV and the IVC (t1, 67.2 ± 8.7 mmHg *vs.* t2, 31.1 ± 3.3 mmHg, $p < 0.001$) and rose to 48.4 ± 4.4 mmHg after unclamping (t3), yet still lower than that at t1 ($p < 0.001$). Group K showed similar trend in the MAP with Group C. In Group K, the MAP was 60.2 ± 7.9 mmHg at t1, 27.3 ± 6.2 mmHg at t2, and 49.8 ± 5.8 mmHg at t3 ($p < 0.001$, compared with t1) (Fig. 2). Before clamping, the PRs in Group C and Group K were 277 ± 12 bpm and 264 ± 27 bpm, respectively, and they significantly decreased to 208 ± 24 bpm and 199 ± 11 bpm after clamping ($p < 0.001$). After unclamping, PRs in Group C and Group K returned to 256 ± 10 bpm and 248 ± 31 bpm, respectively, significantly higher than those after clamping ($p < 0.001$), yet showing no significant difference with those before clamping ($p = 0.185$) (Fig. 3).

3.4. Quantity of TNF-α mRNA and ICAM-1 mRNA in lung tissue

Group C showed the highest quantity of TNF-α mRNA and ICAM-1 mRNA among all groups (TNF-α mRNA Group S *vs.* Group C $p = 0.004$, Group C *vs.* Group K $p < 0.001$ and ICAM-1 mRNA Group S *vs.* Group C $p = 0.035$, Group C *vs.* Group K $p = 0.004$). Group K showed similar quantity of TNF-α mRNA and ICAM-1 mRNA with Group S (TNF-α mRNA $p = 0.082$ and ICAM-1 mRNA $p = 0.923$) (Table 2).

3.5. Expression of NF-κB/p65 in the lung tissue

Expression of NF-κB/p65 in the lung tissue was quantitatively analyzed by Western blot Analysis. Group C showed the highest quantity of NF-κB/p65 among all groups ($p < 0.001$), whereas Group S and Group K showed no significant difference in NF-κB/p65 expression ($p = 0.084$) (Fig. 4, Table 2).

3.6. Test of correlation between quantity of NF-κB/p65 and expression of TNF-α mRNA and ICAM-1 mRNA

Fig. 5 shows the result of Pearson's correlation analysis. The quantity of NF-κB/p65 in lung tissue correlated positively

Table 1
Lung W/D ratio in each group

Group	W/D ratio	*p* value of Tukey test and difference between groups
Group S ($n = 10$)	3.70 ± 0.69	Group C versus Group S, $p < 0.001$, difference = 3.2087
Group C ($n = 10$)	6.91 ± 1.71[a]	Group K versus Group S, $p = 0.009$, difference = 1.7726
Group K (n = 10)	5.47 ± 1.10[a,b]	Group K versus Group C, $p = 0.039$, difference = 1.4360

The W/D ratios were expressed as mean ± standard deviation and were compared by one way analysis of variance, Tukey test.
[a] Group C versus Group S, $p < 0.001$, Group K versus Group S, $p = 0.009$.
[b] Group K versus Group C, $p = 0.039$.
Group C = control group; Group K = ketamine group; Group S = sham group.

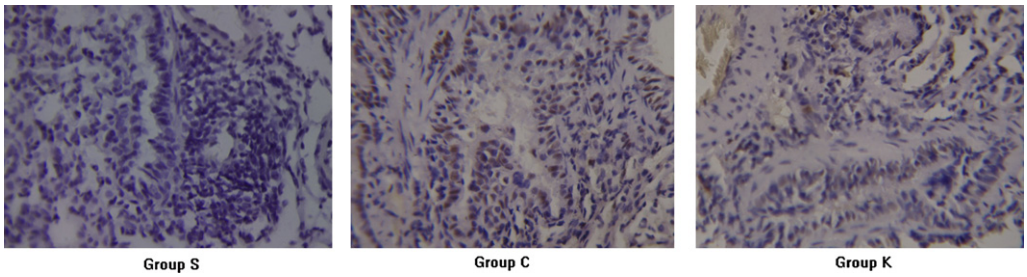

Fig. 1. Immunohistochemical stain of the lung tissue after hepatic ischemia-reperfusion induced lung injury. Sections were 4–6 μm thick. Three areas were chosen for evaluation in each hematoxylin and eosin stained section with a scope of 100 μm. Intracellular adhesion molecule (ICAM)-1 was stained brown by enzyme-linked immunosorbent assay stain in the lung tissue. ICAM-1 was found in Group C and Group K, but not in Group S. ICAM staining was of higher density and intensity in Group C than in Group K. The polymorphonuclear neutrophils in the pulmonary interstitium were stained blue. Group C showed the highest density of polymorphonuclear neutrophils among the three groups. Group C = control group; Group K = ketamine group; Group S = sham group.

with the expression of TNF-α mRNA (correlation coefficient, R = 0.849, $p < 0.001$) and ICAM-1 mRNA (R = 0.639, $p < 0.001$) (Fig. 4).

4. Discussion

Major liver surgeries such as OLT often require cross-clamping of the IVC and the HPV to reduce excessive blood loss and improve surgical condition. However, this procedure also causes acute lung injury of various severity due to hypotension and release of inflammatory mediators induced by ischemia-reperfusion.[1,2,11] The lung is one of the most susceptible targets for remote organ damage during a systemic inflammatory response. Acute lung vascular injury resembling features of human acute respiratory distress syndrome (ARDS) was reported in experimental models of ischemia-reperfusion of the liver.[12] Although liver does bear more-evident tissue injury after the HIR, in the clinical setting, postoperative lung injury is now a tough problem we are facing after the OLT, when the transplanted liver functions well. So in this study, we focused on the protective effect of small dose of ketamine on lung injury following the HIR and its possible mechanism.

In this study, the IVC and the HPV of rabbits were clamped with noninvasive bulldog clamps and unclamped 60 min later to simulate HIR. Our study demonstrates: 1) the HIR caused a remote acute lung injury, as demonstrated by the changes of lung histology and W/D ratio, the infiltration and aggregation of neutrophils, and the production of proinflammatory mediators such as TNF-α, ICAM-1, and NF-κB/p65. 2) 0.5 mg/kg ketamine pretreatment did not prevent the change of the MAP and the PR caused by the HIR, but it did attenuate pulmonary edema and lung injury; meanwhile, it did inhibit aggregation of neutrophils and production of proinflammatory mediators such as TNF-α, ICAM-1, and NF-κB/p65. 3) Our data provide further evidence that pretreatment with small-dose ketamine inhibited activation of NF-κB/p65 in rabbit HIR model.

The severity of acute lung injury induced by surgery varies. Mild injury appears as increase of lung water, and severe injury as pulmonary edema and ARDS. Li et al reported that the OLT caused 58.2% acute pulmonary edema, 27.5% severe acute lung injury, and 5.5% ARDS.[13] In the clinical setting, postoperative lung injury is now a tough problem clinicians we are facing after the OLT, when the transplanted liver functions well. The mechanism underlying HIR-induced acute lung

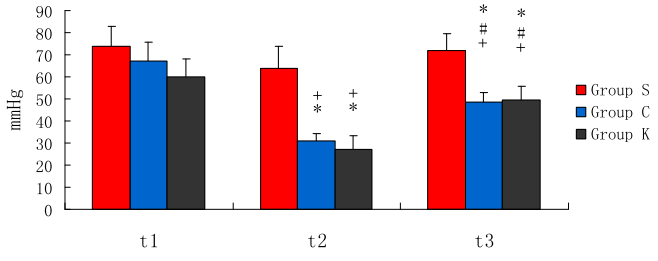

Fig. 2. Mean artery pressure (MAP) changes in each group. The MAP was measured directly by means of ear median artery (mmHg), expressed as mean ± standard deviation. Data were analyzed by one way analysis of variance Tukey test. MAPs were recorded at 10 minutes after the exposure of the HPV and the inferior vena cava without clamping (t1), 10 minutes after cross-clamping of the HPV and the inferior vena cava (t2) and 10 minutes after unclamping (t3). In Group C and Group K, MAPs were significantly lower at t2 and t3 than at t1. The MAPs in Group C and Group K were significantly higher at t3 than at t2. Group C and Group K showed significantly lower MAPs at t2 and t3 than did Group S. $^{*}p < 0.05$ compared with t1 within group. $^{\#}p < 0.05$ compared with t2 within group. $^{+}p < 0.05$ compared with Group S. Group C = control group; Group K = ketamine group; Group S = sham group.

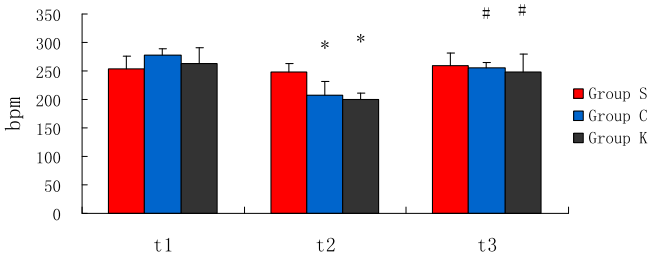

Fig. 3. Pulse rate (PR) changes in each group. The PRs were measured by count of arterial waveform (bpm) expressed as mean ± standard deviation data were analyzed by one way analysis of variance Tukey test. Group S showed no significant PR change during the whole procedure ($p > 0.05$). In Group C and Group K, PR decreased significantly at t2 and increased significantly at t3 ($p < 0.05$). All groups showed the similar PR at t3 ($p > 0.05$). $^{*}p < 0.05$ compared with t1 within group, $^{\#}p < 0.05$ compared with t2 within group. Group C = control group; Group K = ketamine group; Group S = sham group.

Table 2
Quantity of TNF-α mRNA, ICAM-1 mRNA, and NF-κB/p65 in lung tissue

	TNF-α mRNA	ICAM-1 mRNA	NF-κB/p65 (pg/mL, $n = 10$)
Group S	1.46 ± 0.24* ($p < 0.001$)	0.95 ± 0.18* ($p = 0.005$)	0.36 ± 0.08* ($p < 0.001$)
Group C	2.43 ± 0.41	1.33 ± 0.33	0.66 ± 0.06
Group K	1.83 ± 0.44* ($p = 0.006$)	0.99 ± 0.16* ($p = 0.009$)	0.45 ± 0.10* ($p < 0.001$)

Data are expressed as mean ± standard deviation, compared by one way analysis of variance Tukey test.
The expression of TNF-α mRNA and ICAM-1 mRNA was detected by real-time quantitative polymerase chain reaction, with a copy number of 30 and glyceraldehyde 3-phosphate dehydrogenase as reference. Expression of NF-κB/p65 was detected by Western blot.
* A p value <0.05 compared with Group C.
Group C = control group; Group K = ketamine group; Group S = sham group; ICAM = intracellular adhesion molecule; NF-κB = nuclear factor kappa B; TNF = tumor necrosis factor.

injury may be related to hypotension induced by cross-clamping of the IVC and the HPV.[14,15] Our study showed that the lung W/D ratio, an important index for pulmonary edema, was higher in both Group C and Group K than in Group S, indicating increased lung water caused by HIR. Furthermore, the W/D ratio in Group K was lower than in Group C ($p < 0.05$), implying that 0.5 mg/kg ketamine pretreatment before the HPV clamping and the IVC clamping might attenuate the pulmonary edema induced by the HIR. It is noteworthy that ketamine pretreatment could not prevent the decreased MAP and PR during the HIR as assumed, suggesting that ketamine alleviated lung injury following the HIR by means of mechanisms other than affecting hemodynamic parameters.

To further explore the underlying mechanisms by which ketamine alleviated lung injury following the HIR, we investigated its effect on production of pulmonary TNF-α and ICAM-1. TNF-α activates the inflammatory cascade by initiating the production of several cytokines and chemokines and increasing expression of endothelial adhesion molecules such as ICAM-1, in vascular endothelial cells that promote neutrophil adherence and aggregation.[16] It has been reported that upregulation of TNF-α and ICAM-1 are positively related

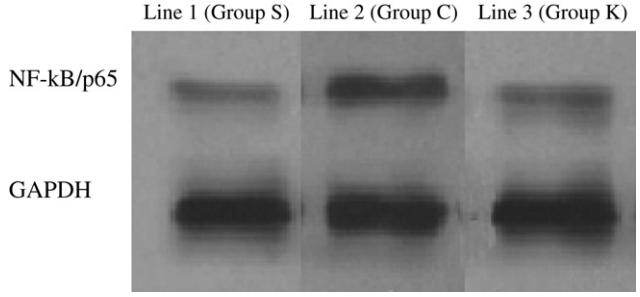

Fig. 4. Expression of NF-κB/p65 detected by Western blot. Line 1 stands for Group S, Line 2 for Group C and Line 3 for Group K. Expression of NF- κB/p65 was detected by Western blot, with GAPHD as reference. Intracellular expression of NF-κB/p65 was quantitatively expressed as NF-κB/p65/GAPDH. GAPDH = glyceraldehyde 3-phosphate dehydrogenase; Group C = control group; Group K = ketamine group; Group S = sham group; NF-κB = nuclear factor kappa B.

to severity of lung injury.[17,18] Using lipopolysaccharide-induced acute lung injury model in Sprague-Dawley rat, Sun et al confirmed that 50 mg/kg ketamine could inhibit upregulation of TNF-α mRNA in lung tissue, whereas a small-dose of ketamine (0.5 mg/kg) could not.[19,20] However, Pandey et al have reported that intramuscularly injected large-dose ketamine could cause pulmonary artery hypertension and acute pulmonary edema, indicating a potential unfavorable effect of ketamine despite its favorable effect in inhibiting the production of proinflammatory cytokines.[10] This unfavorable effect limits the use of ketamine in liver surgeries such as the OLT, though ketamine may alleviate hypotension induced by the HIR. Anyway, such adverse effect of ketamine is dose-dependent. Lewis et al demonstrated that ketamine had an anti-inflammatory activity at a plasma concentration of only 0.1 μg/mL, whereas normal clinical concentration is 2 μg/mL.[21] 0.5 mg/kg ketamine, given intravenously, may reach a plasma concentration close to 0.1 μg/mL. So in the present study, we investigated whether 0.5 mg/kg ketamine has a protective effect on the HIR-induced lung injury and if so its underlying mechanism.

In this study, pretreatment with 0.5 mg/kg ketamine was given intravenously 10 minutes before cross-clamping of the HPV and the IVC. We found that this strategy inhibited the upregulation of proinflammatory cytokines and attenuated pulmonary edema, which was consistent with suppression of neutrophil accumulation in the lung; however, we did not find that ketamine pretreatment could prevent hypotension and increase heart rate after clamping of the HPV and the IVC. Kozhevnikov et al proposed that in the OLT, ketamine could alleviate hypotension after unclamping of the IVC and the HPV, and thus attenuate pulmonary edema.[22] In this study, we did not find any beneficial effect of 0.5 mg/kg ketamine pretreatment on the MAP and the PR, suggesting that the protective effect of ketamine pretreatment is more likely by means of inhibition of upregulation of inflammatory mediator synthesis.

Compared with the sustained increase of TNF-α and long-lasting stimulation of toxins induced by severe infection,[23] hypotension and ischemia-reperfusion injury induced by liver surgery only lasts a short time. Furthermore, cross-clamping of the ICV and the HPV impedes hepatic metabolism of ketamine, resulting in prolonged effects of ketamine. This is probably why 0.5 mg/kg ketamine pretreatment could reduce production of proinflammatory cytokines such as TNF-α and ICAM-1, and attenuate pulmonary edema.

As one of the crucial transcription factors that modulate and control the transcription of a series of proinflammatory cytokines, NF-κB might be activated and involved in the remote lung injury following HIR.[4,8,24,25] It has been assumed that ketamine might act at the transcriptional level, at least in part, by means of inhibition of NF-κB, to reduce production of proinflammatory cytokines. Our experiment further confirms this assumption. Our study showed that quantity of TNF-α mRNA and ICAM-1 mRNA in lung tissue following the HIR positively correlated with the content of NF-κB/p65, one of the important functional subunits of NF-κB family.[26] Our results also demonstrated that

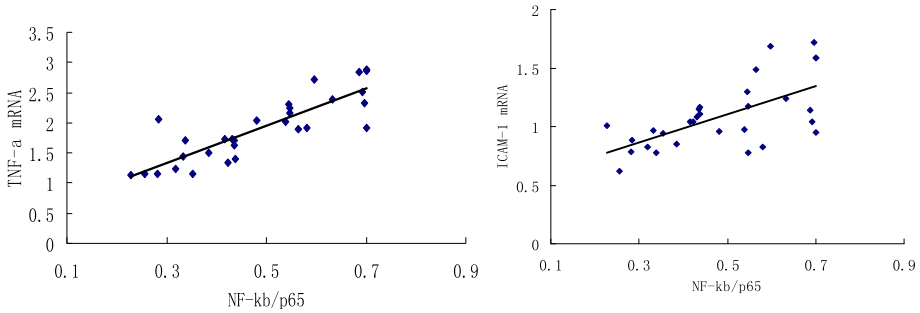

Fig. 5. Pearson correlation test of the total expression of TNF-a mRNA, ICAM-1 mRNA, and NF-κB/p65. Correlation coefficient of TNF-α mRNA and NF-κB/p65 is R = 0.849, significant in two-tailed test ($n = 30$, $p < 0.001$), correlation coefficient of ICAM-1 mRNA and NF-κB/p65 is R = 0.639, significant in two-tailed test ($n = 30$, $p < 0.001$). ICMA = intracellular adhesion molecule; NF-κB = nuclear factor kappa B; TNF = tumor necrosis factor.

ketamine pretreatment resulted in decrease of TNF-α mRNA and ICAM-1 mRNA, which correlated with reduction of NF-κB/p65, indicating that NF-κB/p65 mediates the synthesis and expression of TNF-α mRNA and ICAM-1 mRNA.

N-methyl-D-aspartate receptors (NMDARs) widely exist in neurons; however, recent studies have revealed that NMDARs also exist in other cells such as intestinal cells and pancreatic cells. Wang et al recently demonstrated that endogenous glutamate mediated hypoxia-induced newborn rat lung injury by means of NMDARs,[27] suggesting pulmonary existence of the NMDARs, which may induce anti-inflammatory effects. It is possible that ketamine, as an antagonist of the NMDARs, may mediate NF-κB pathway by means of NMDARs to reduce production of proinflammatory cytokines, resulting in relief of lung injury. Further studies are necessary to investigate this possibility.

In conclusion, during the HIR, clamping and unclamping of the IVC and the HPV may result in severe hypotension, acute pulmonary edema, and lung injury. Pretreatment with 0.5 mg/kg ketamine 10 minutes before clamping of the IVC and the HPV did not attenuate hypotension during the HIR, nor did it totally prevent pulmanory edema induced by the HIR in rabbits. However, 0.5 mg/kg ketamine pretreatment did significantly attenuate pulmonary inflammatory response and pulmonary edema to a certain extent. Furthermore, ketamine pretreatment did inhibit upregulation of TNF-α mRNA and ICAM-1 mRNA and aggregation of PMNs in lung tissue, which may be mediated by NF-κB pathway. Our findings indicate that 0.5 mg/kg ketamine pretreatment shows a protective effect on acute lung injury induced by the HIR.

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