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Low-dose ketamine pretreatment reduces oxidative damage and inflammatory response following CO₂ pneumoperitoneum in rats

Abstract

Purpose: The duration of pneumoperitoneum during laparoscopic procedures may contribute to post-surgical oxidative stress. Previous studies have shown that low-dose ketamine, an anesthetic with anti-inflammatory properties, protects various organs from ischemia-reperfusion injury. This study investigated the effects of low-dose ketamine on the overproduction of oxidants and the tissue damage caused by intra-abdominal pressure during CO₂ pneumoperitoneum.

Methods: Male Sprague Dawley rats received a CO₂ pneumoperitoneum of 15 mmHg and preceded by either low-dose ketamine (KP1, 5 mg/kg; KP2, 10 mg/kg) or 0.9% saline (PR, 3 ml). General anesthesia was provided by pentobarbital and sevoflurane. The control group (CR) received an intraperitoneal saline injection and sham surgery. Three hours after pneumoperitoneum, serum concentrations of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), malondialdehyde (MDA), superoxide dismutase (SOD) and intestinal fatty acid binding protein (iFABP) were measured and liver, kidney, lung, and intestine were evaluated for tissue damage.

Results: The highest plasma MDA, TNF- α , IL-6 and iFABP values were observed at T1 (after 3 hours of pneumoperitoneum) in the PR group, followed by the KP1, KP2, and CR groups ($P < 0.01$). SOD concentrations showed an opposite trend and were highest in the CR group, followed by the KP2, KP1, and PR groups ($P < 0.01$). TNF- α concentration was significantly lower in the KP2 than the KP1 group ($P < 0.05$). Histopathologic scoring of organ sections demonstrated the lowest scores in the KP2 group, followed by the KP1 and PR groups, in an increasing order ($P < 0.05$).

Conclusion: Pretreatment with low-dose ketamine before general anaesthesia protects against potential oxidative damage and inflammatory response caused by CO₂ pneumoperitoneum.

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Manuscript submitted 3rd October, 2013
Manuscript accepted 3rd April, 2014

Clin Invest Med 2014; 37 (3): E124-E130.

As a result of increased expertise in technically demanding procedures and improvements in instrumentation, laparoscopic surgery has become an integral part of modern-day surgical practice [1]. Despite the general safety of the procedure, ischemia and other adverse effects may occur after routine laparoscopy, even in young, healthy patients with no preoperative complications [2,3]. A recent review of animal studies and human clinical trials indicated that pneumoperitoneum decreases splanchnic perfusion, with a concomitant increase in oxidative stress [4]. Many studies have focused on changes in blood flow in intra- and extra-peritoneal organs, which is reduced during operation but is normalized after desufflation [5-7]. This ischemia/reperfusion effect leads to the overproduction of oxidants or the malfunction of the scavenging systems, postoperatively; even causing cellular damage and resultant organ dysfunction [8,9,10].

Ketamine is an *N*-methyl-D-aspartate (NMDA) receptor antagonist that has been extensively studied and is used as a safe and adequate anesthetic in clinical practice [11]. When administered intravenously in rats, low-dose ketamine exhibits a dose-dependent inhibition of the elevation in proinflammatory cytokines such as TNF- α and IL-6 that is associated with lipopolysaccharide-induced injury [12]. Because of its novel analgesic and anti-inflammatory properties, ketamine has been specifically recommended for use in situations characterized by inflammation.

There are few studies on the effects of ketamine on potential organ ischemic threat after carbon dioxide (CO₂) pneumoperitoneum. The purpose of the present study was to evaluate whether ketamine preconditioning exerts a protective effect by limiting the production of oxidants associated with pneumoperitoneum in rats. To accomplish this, a low dose of ketamine was administered to rats prior to pneumoperitoneum and the subsequent changes in expression of systemic oxidants and intestinal inflammatory cytokines and tissue damage were assessed.

Materials and Methods

Experimental animals

Forty adult male Sprague Dawley rats, weighing 200-250 g were obtained from the Animal Center of Jinling Hospital, Nanjing China. Animals were maintained under standard conditions, including stable room temperature (25 $\pm$ 2°C), 12:12-h light:dark cycle and access to commercial rat pellets and water *ad libitum*. The study was approved by the Ethics Committee of the School of Medicine, Nanjing University.

Induction of Pneumoperitoneum

After 12 h of fasting, the rats were divided into four groups for study:

- 1) control group (CR, n=10);
- 2) pneumoperitoneum control group (PR, n=10);
- 3) pneumoperitoneum with ketamine at 5 mg/kg group (KP1, n=10);
- 4) pneumoperitoneum with ketamine at 10 mg/kg group (KP2, n=10).

Animals in the CR group received an intraperitoneal injection of 3 mL 0.9% saline solution at time T0 and underwent sham surgery; those in the PR group were injected intraperitoneally with 3 mL 0.9% saline intraperitoneally at T0, followed by a 3 h CO₂ pneumoperitoneum; and rats in the KP1 and KP2 groups were injected intraperitoneally with 5 mg/kg and 10 mg/kg ketamine (2ml/100mg, Gutian China), respectively, at T0, also followed by a 3 h CO₂ pneumoperitoneum.

For all four groups, all animals received 40 mg/kg of 3% pentobarbital for induction of anesthesia, followed by a lower dose of pentobarbital (approximately 25% of the original dose) and sevoflurane for maintenance of anesthesia. The rats then underwent a small umbilical incision followed by a purse-string suture to fixate an 18-gauge cannula in the abdominal cavity, through which CO₂ was insufflated into the peritoneal cavity (rats that underwent sham surgery had cannula inserted but no CO₂ was insufflated) [13]. Insufflation was monitored by a sphygmomanometer and oxygen monitor (GME-300, Abbott) to maintain a constant intra-abdominal pressure of 15 mmHg, and a normal blood pH range. Insufflation was maintained for 3 h and the end time was defined as T1. T1 was also defined the end of 3 h sham surgery in CR groups.

MDA, SOD, IL-6 and TNF- α Determinations

One millilitre of venous blood was obtained from each rat at times T0 and T1, after which the rats were sacrificed with an intracardiac potassium injection while still anesthetized. After centrifugation at 10,000 $\times$ g for 15 min at 4°C, serum was removed and concentrations of interleukin (IL)-6 and tumor necrosis factor (TNF)- α were determined using enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA) and serum concentrations of malondialdehyde (MDA) and superoxide dismutase (SOD) were measured with corresponding assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Absorbance at 450 nm was determined using the Tecan Sunrise microplate reader (Unterberg Strasse

TABLE 1. Histopathological evaluation of tissues

(a) Hauet's histopathologic description and grading score of liver, lung, kidney and intestinal samples

Score	% of abnormality
1	No abnormality
2	Mild lesions affecting 10% of samples
3	Lesions affecting 25% of samples
4	Lesions affecting 50% of samples
5	Lesions affecting more than 75% of samples

(b) Histopathological evaluation of tissues in different groups (mean±SD)

Variables	PR(n=10)	KP1(n=10)	KP2(n=10)
Liver	7.3±1.8	7.0±0.5	5.2±2.2 <i>a</i>
Lung	5.8±2.2	5.8±2.4	5.4±1.6
Kidney	9.3±2.1	8.2±2.1	5.9±1.8 <i>a</i>
Intestine	9.0±2.4	7.3±1.5 <i>a</i>	6.2±2.0 <i>a</i>

The mean±SD values of the total tissue damage are displayed. *a* denote the significant P values (< 0.05) for the comparisons with PR group.

IA, Austria) in accordance with the manufacturer's instructions. The results were expressed as $\mu\text{mol/L}$ for MDA, U/mL for SOD, pg/mL for TNF- α and IL-6 and ng/mL for iFABP.

Histopathologic Examination

For each rat, samples of liver, kidney, lung and intestinal tissue were preserved in buffered-formalin solution and embedded in paraffin. Histopathologic sections were stained with hematoxylin and eosin staining. A pathologist, who was blinded to the study group assignment, examined the sections under light microscopy for tissue injury. The histologic scoring system de-

scribed by Hauet *et al.* [1,14] was used (Table 1a). Each section was assessed for inflammatory cell infiltration, cellular edema, congestion and hemorrhage. For each tissue, total damage was calculated as the sum of the scores for intracellular edema, congestion, interstitial hemorrhage, and inflammatory cell infiltration parameters, according to Hauet's injury grading scale.

Statistics Analysis

Statistical analyses of the data were performed using SPSS 11.0 for Windows software on a personal computer. All data are presented as mean $\pm$ standard deviation. Statistical analysis of oxidant and cytokine data was performed using one-way analysis of variance, followed by least square difference *post hoc* tests. Differences between groups were considered statistically significant if the P value was less than 0.05.

Results

The TNF- α , IL-6, MDA, SOD, and iFABP values for the T0 and T1 time points are shown in Table 2 and Figures 1 to 4. The serum TNF- α and IL-6 values at T1 were significantly higher in the PR than the CR group ($P < 0.01$). For both the KP1 and KP2 groups, the TNF- α and IL-6 serum levels were higher than in the CR group ($P < 0.01$) but lower than in the PR group ($P < 0.01$) at T1. The TNF- α concentration at T1 was significantly lower in the KP2 group than in the KP1 group ($P < 0.05$, Fig. 1, Fig. 2), whereas the IL-6 level at T1 was similar for the KP1 and KP2 groups.

The lowest MDA values at T1 were observed in the CR group, followed in increasing order by the KP2, KP1 and PR groups ($P < 0.01$) (Fig. 3).

The serum SOD concentration at T1 was significantly lower in the PR group than the CR group ($P < 0.01$). The SOD concentration at T1 was higher in the KP1 and KP2

TABLE 2. Plasma oxidant and cytokines in RC, PC, KP1, KP2 in rats at T1 (mean±SD)

	CR	PR	KP1	KP2	P-value
MDA ($\mu\text{mol/L}$)	1.93±0.24	4.31±0.35	3.80±0.29 ^a	3.66±0.25 ^a	$P < 0.01$
SOD (U/mL)	6.10±0.26	3.64±0.18	4.16±0.17 ^a	4.33±0.25 ^a	$P < 0.01$
IL-6 (pg/mL)	17.81±2.81	56.45±5.80	41.53±5.38 ^a	37.27±4.50 ^a	$P < 0.01$
TNF- α (pg/mL)	9.51±0.34	15.57±1.02	12.91±0.69 ^a	11.57±0.62 ^{ab}	$P < 0.01$
iFABP (ng/mL)	25.54±4.43	44.39±11.48	39.92±9.72 ^a	36.46±11.00 ^a	$P < 0.01$

T1 – end of 3 hours of sham operation or pneumoperitoneum

MDA, malondialdehyde; TNF- α , tumor necrosis factor-alpha; IL-6, interleukin-6; SOD, superoxide dismutase; iFABP, fatty acid binding protein.

^a Compared with PR control, $P < 0.05$ (LSD *post hoc* test).

^b Compared with KP1, $P < 0.05$ (LSD *post hoc* test).

FIGURE 1. Serum IL-6 levels in T0 and T1 of CR, PR, KP1 and KP2 groups. Values are the means and 95% confidence interval of IL-6.

FIGURE 2. Serum TNF-α levels in T0 and T1 of CR, PR, KP1 and KP2 groups. Values are the means and 95% confidence interval of TNF-α.

FIGURE 3. Serum MDA levels in T0 and T1 of CR, PR, KP1 and KP2 groups. Values are the means and 95% confidence interval of MDA.

FIGURE 4. Serum SOD levels in T0 and T1 of CR, PR, KP1 and KP2 groups. Values are the means and 95% confidence interval of SOD.

groups than in the PR group ($P < 0.01$), but lower than that in the CR group (Fig. 4).

The pattern of serum iFABP levels at T1 was similar to that observed for MDA. The iFABP concentration was lowest in the CR group, followed in increasing order by the KP2, KP1, and PR groups ($P < 0.01$). The iFABP levels in the KP2 and KP1 groups were significantly lower than in the PR group ($P < 0.01$), but higher than in the CR group.

The total tissue damage under light microscopy revealed that in KP2 group was significantly lower than in KP1 and PR groups for liver, kidney and intestine ($P < 0.05$), but not for lung, which also showed a decrease trend (Tab 1a and b). The amount of lung damage was also lowest in the KP2 group. Total damage was calculated as the sum of total of the scores for each of the intracellular edema, congestion, interstitial hemorrhage, and inflammatory cell infiltration parameters of the histologically examined [1].

Discussion

CO₂ pneumoperitoneum is widely used in laparoscopy and patients now routinely undergo laparoscopic operations of 2 h or longer without apparent intestinal ischemia or other adverse consequences; however, the probability of occurrence of minor mucosal ischemic injury, and possibly even more potential damage, is increased after pneumoperitoneum. To provide adequate visualization and operating space, laparoscopic surgery usually requires the use of an intra-abdominal pressure of 12–15 mmHg, which is higher than the portal circulation pressure of 7–10 mmHg. Furthermore, the gastrointestinal tract is particularly sensitive to ischemia and hypoxia [15]. In addition, deflation of the abdomen at the end of the procedure leads to reperfusion of hypoperfused organs, which may worsen damage due to harmful oxidative effects [16]. Thus, although this is usually not clinically apparent, organ ischemia in pneumoperitoneum should not be ignored.

Various preconditioning methods to minimize organ or cellular injury and dysfunction associated with pneumoperitoneum have been investigated [3,17]. The commonly-used anesthetic, ketamine, has been found to act in a dose-dependent manner; low doses have beneficial sedative, analgesic, and anti-inflammatory effects, whereas large doses may cause adverse reactions such as irritability and hallucinations [11,18]. Structure of benzene may play a protective role in scavenging free radicals and inhibiting the activity of inflammatory cytokines. In our study, a single intraperitoneal injection of ketamine (5 mg/kg or 10 mg/kg) was administered prior to the initiation of pneumoperitoneum. This is lower than the conventional dosage [19]. For this reason, an attenuated laparoscopy-

induced injury was found. This reduced injury might be attributed to ketamine's ability to inhibit the production of inflammatory cytokines during pneumoperitoneum, and thus reducing stress response, protecting the intestinal barrier from toxins, antigens invading, and preventing bacterial translocation.

Ketamine has also been demonstrated to inhibit the expression of adhesion molecules on inflammatory cells and reduce leukocyte reactivity *in vivo* and *in vitro* studies [20], and was recently shown to exhibit anti-inflammatory effects on lipopolysaccharide-induced astrocytes by suppressing the activation of nuclear factor- κ B, an important transcription regulatory factor, through reduced expression of TLR4 [21].

TNF- α is among the earliest and most potent mediators of host responses to acute injury or infection. It can promote marked and rapid metabolic changes and the activation of cytokine mediators, such as IL-6. The concentration of circulating IL-6 appears to be proportional to the extent of tissue injury and inflammatory changes during surgery [22]. Single studies on preoperative anesthetics have been widely studied and have indicated anti-inflammatory effects at anesthetic doses. There is evidence that a subanesthetic dosage has beneficial effects on gastrointestinal recovery after surgery due to a potent modulatory effect of the proinflammatory response, and the extent of systemic inflammatory response is associated with the outcome of the intervention. Thus, it has become increasingly evident that both circulating and tissue concentrations of cytokines play important roles in surgery outcome and should be well controlled. Kawamura and Corcoran [23,24] both studied the effects of sevoflurane and propofol on cytokine balance in patients undergoing surgery. They found that sevoflurane and propofol suppressed the production of IL-6 and IL-8, and a change in the balance between pro- and anti-inflammatory cytokines may be one of the most important mechanisms of anesthetic-induced tissue protection.

In our study, changes in levels of cytokines were investigated to assess the severity of inflammation. Animals in the PR, KP1, and KP2 groups had produced more IL-6 and TNF- α by T1 than the CR group, suggesting that intraperitoneal insufflation of CO₂ gas for a period of 3 h led to a substantial inflammatory response and/or tissue damage in rats. The higher IL-6 and TNF- α levels in the PR group, compared with the KP1 and KP2 groups, demonstrate that ketamine pretreatment reduced the inflammatory reaction or tissue ischemia. Similarly, the observation that MDA concentration was highest in the PR group, followed by the KP1, KP2, and CR groups in descending order, suggests that ketamine may effectively inhibit the release of oxygen free radicals such as MDA, and thereby reduce the cellular disruptions that are induced by CO₂ pneu-

moperitoneum. In contrast, we found an opposite trend for the antioxidant marker; the SOD concentration at T1 was highest in the CR group, followed in descending order by the KP2, KP1, and PR groups. SOD is a ubiquitous antioxidant enzyme in aerobic organisms. An imbalance between oxidants and antioxidants in favor of oxidants can lead to organ dysfunction [25]; therefore, high levels of oxidative stress and inflammatory cytokine response markers (MDA, TNF- α and IL-6) and low levels of antioxidant markers (SOD) in the PR group, compared with KP1 and KP2 groups, suggest that oxidative tissue damage was more pronounced in the PR group. The lower TNF- α concentration in the KP2 group in comparison with the KP1 group reveals that a higher dose of ketamine for pretreatment before CO₂ pneumoperitoneum may be more protective than a lower dose.

iFABP is a sensitive biochemical marker of early intestinal mucosal injury and results from mesenteric ischemia [26]. The concentration of iFABP in the KP1 and KP2 groups at T1 was significantly higher than in the CR group but lower than that in the PR group. These results are consistent with the changes in levels of TNF- α and IL-6, which indicates that low-dose ketamine pretreatment inhibited the release of pro-inflammatory cytokines and reduced the accumulation of polymorphonuclear leukocytes in tissues. Ketamine protected the intestinal mucosal and attenuated the injury.

Pathology is an objective indicator of organ damage. The lower scoring of total tissue damage in KP groups reveals a mild lesion, and the higher ketaminedose (10 mg/kg) seemed to provide better organ protection, without increasing blood pressure or otherwise stimulating the sympathetic nervous system.

There are limitations to our study design and conclusions. A single 15 min interval was used between the injection of ketamine and the induction of general anesthesia, and only two ketamine concentration were studied. Additional investigations are warranted to determine the optimum time and the most appropriate dose of ketamine administration. Another condition with elevated intra-abdominal pressure, abdominal compartment syndrome, is known to increase intracranial hypertension and promote the release of vasopressin. Therefore, it is possible that at least some of the observed protective effects were related to the ability of low-dose ketamine to lower intracranial hypertension and thus reduce the release of vasopressin, a potent intestinal vasoconstrictor.

Despite its limitation, this study does suggest that low-dose ketamine pretreatment can reduce oxidative stress and the inflammatory cytokine response associated with 3 h maintenance of a high intra-abdominal pressure in rats, which are in

accordance with data from a recent meta-analysis showing that ketamine significantly inhibits the early postoperative inflammatory response [27].

The results of our animal study still need to be verified in humans to make safe clinical conclusions; however, they give us new insight into mechanisms for protection against the ischemia-reperfusion damage induced by prolonged pneumoperitoneum.

Acknowledgments

The work was supported by grants from "Natural Science Foundation of Jiangsu Province"(No. BK2008237) and "Nanjing Science and Technology Development Plan"(No. 201104027). We thank Nie Yao, Zhu Chuanrong and Liu Xingdong for their encouragement and support; Zhao Xiaofan, Shen Bo, He Qin, Wang Jian and Xu Yuqin for technical support in the laboratory of Jinling Hospital.

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