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Large dose ketamine inhibits lipopolysaccharide-induced acute lung injury in rats

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Abstract. *Background:* Sepsis is associated with the highest risk of progression to acute lung injury or the acute respiratory distress syndrome. Ketamine has been advocated for anesthesia in endotoxemic and other severely ill patients because it is a cardiovascular stimulant. Our study was designed to investigate the effect of ketamine on the endotoxin-induced acute lung injury in vivo.
Materials and methods: Adult male Wistar rats were randomly divided into 6 groups: saline controls; rats challenged with endotoxin (5 mg/kg) and treated with saline; challenged with endotoxin (5 mg/kg) and treated with ketamine (0.5 mg/kg); challenged with endotoxin (5 mg/kg) and treated with ketamine (5 mg/kg); challenged with endotoxin (5 mg/kg) and treated with ketamine (50 mg/kg); saline injected and treated with ketamine (50 mg/kg). TNF-α, IL-6 and NF-kappa B were investigated in the tissues of the lung after 2 h. Myeloperoxidase (MPO) activity and wet/dry weight ratio were investigated 6 h later.
Results: We demonstrated that intravenous administration of endotoxin could provoke significant lung injury, which was characterized by increase of MPO activity and wet/dry weight ratio, TNF-α and IL-6 expression and NF-kappa B activation. Ketamine (5, 50 mg/kg) inhibited endotoxin-induced NF-kappa B activation. Ketamine only at a dose of 50 mg/kg inhibited TNF-α and IL-6 production, and decreased MPO activity and wet/dry weight ratio after endotoxin challenge.
Conclusions: Ketamine, only at a supra-anesthetic dosage, could inhibit endotoxin-induced pulmonary inflammation in vivo.

Key words: Endotoxin – NF-kappa B – TNF-α – Lung – Ketamine – Rats

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Introduction

Acute respiratory distress syndrome (ARDS) is a common, devastating clinical syndrome of acute lung injury (ALI) that affects both medical and surgical patients. Until recently, most studies of acute lung injury and the acute respiratory distress syndrome have reported a mortality rate of 40 to 60 percent [1–3].

Sepsis is associated with the highest risk of progression to acute lung injury or the acute respiratory distress syndrome, at approximately 40 percent [4]. The intravenous anesthetic ketamine has been advocated for use in endotoxemic or severely ill patients because of its stimulatory effects on the cardiovascular system [5]. Further it also suppresses lipopolysaccharide (LPS)-induced tumor necrosis factor alpha (TNF-α) production at concentrations > 20 μg/mL, and significantly suppresses both LPS-induced and TNF-induced interleukin 6 (IL-6) and IL-8 production at concentrations > 100 μg/mL in human whole blood in vitro [6]. It is also demonstrated that ketamine has the ability to suppress LPS-induced TNF-α and NF-kappa B activation in peripheral blood mononuclear cells (PBMC) [7]. Since TNF-α, IL-6, and NF-kappa B are known to be very important inflammatory mediators in the pathogenesis of LPS-induced acute lung injury [8], and ketamine seems to have a powerful inhibitory effect on these inflammatory mediators, therefore this study was intended to investigate whether ketamine could suppress endotoxin-induced acute lung injury in vivo.

Materials and methods

Animals and experiment protocol

Adult male Wistar rats (50 days old), which were free of pathogens, were purchased from Shanghai Animal Center, Shanghai, China. The rats were exposed daily to 12 h of light and 12 h of darkness. Rodent food and water were provided freely. The experimental protocol followed institutional criteria for the care and use of laboratory animals in research.

The animals were anesthetized with urethane (1g/kg) intraperitoneally. Endotoxemia model was established by injection with a dose of lipopolysaccharide (LPS) (5 mg/kg) (Escherichia coli O111: B4, Sigma USA) via the tail vein. Since intravenous administration of LPS (5 mg/kg) was verified to induce significant lung injury in rats [9]. All animals were then treated immediately with ketamine (0.5, 5, 50 mg/kg) (Ketamine Hydrochloride, Hengrui Inc., China) or 0.9% NaCl intraperitoneally. Two or six hours after LPS, animals were exsanguinated. Tissues from the lung were removed and washed with 0.9% NaCl. NF-kappa B, TNF-α and interleukin 6 (IL-6) were investigated two hours after sepsis. Pulmonary edema and lung neutrophil accumulation were investigated six hours after sepsis. We used six rats in *each time point of each group.*

Analytical methods

Nuclear protein extraction. Nuclear extracts of the lung tissue were prepared by hypotonic lysis followed by high salt extraction [10, 11]. In brief, ~0.1g of frozen tissue was homogenized in 0.8 ml ice-cold buffer A, composed of 10 mM HEPES (pH 7.9), 10 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 1.0 mM dithiothreitol (DTT), and 0.5 mM phenylmenthysulfonylfluoride (PMSF) (all from Sigma Chemical Co.). The homogenate was incubated on ice for 20 min, after which 50 µl of 10% Nonidet P-40 solution was added (Sigma Chemical Co.); the mixture was vortexed for 30 sec and centrifuged for 1 min at 5000g at 4 °C. The crude nuclear pellet was resuspended in 200 µl of buffer B, containing 20 mM HEPES (pH 7.9), 420 mM NaCl, 1.5 mM $MgCl_2$, 0.1 mM EDTA, 1mM DTT, 0.5 mM PMSF, 25% (v/v) glycerol, and incubated on ice 30 min with intermittent mixing. The suspension was centrifuged at 12,000 g at 4 °C for 15 min. The supernatant containing nuclear proteins was collected and kept at –70 °C for use. Protein concentration was based on Bradford protein assay formats.

Electrophoretic mobility shift assay (EMSA). EMSA was performed using a commercial kit (Gel Shift Assay System; Promega, Madison, WI) as previously described [12]. The NF-kappa B oligonucleotide probe (5′-AGTTGAGGGGACTTTCCCAGGC-3′) was end-labeled with [γ-^{32}P] ATP (Free Biotech, Beijing, China) with T4-polynucleotide kinase. Nuclear protein (80 µg) was preincubated in 9 µl of a binding buffer, consisting of 10 mM Tris-Cl, pH 7.5, 1 mM $MgCl_2$, 50 mM NaCl, 0.5 mM EDTA, 0.5 mM DTT, 4% glycerol, and 0.05g · L^{-1} of poly-(deoxyinosinic deoxycytidylic acid) for 15 min at room temperature. After addition of the 1 µl ^{32}P-labelled oligonuleotide probe, the incubation was continued for 30 min at room temperature. The reaction was stopped by adding 1 µl of gel loading buffer, and the mixture was subjected to non-denaturing 4% polyacrylamide gel electrophoresis in 0.5×TBE buffer. The gel was vacuum-dried and exposed to X-ray film (Fuji Hyperfilm) at –70 °C.

Enzyme-linked immunoassay. TNF-α and IL-6 in the lung were measured using commercially available enzyme-linked immunoassay Kits (Diaclone for TNF-α, Biosource for IL-6) according to the test protocol. Values were expressed as pg per milligram protein (pg/mg prot).

Assessment of lung neutrophil accumulation. Lung myeloperoxidase (MPO) activity was determined as an index of tissue neutrophils accumulation. To measure tissue MPO activity, frozen lungs were thawed and extracted for MPO, following the homogenization and sonication procedure as described previously [13]. MPO activity in supernatant was measured and calculated from the absorbance (at 460 nm) changes resulting from decomposition of H_2O_2 in the presence of o-dianisidine.

Assessment of pulmonary edema. Pulmonary edema was estimated by the wet/dry lung weight ratio, a technique commonly used for assessment of experimental lung injury [14]. Briefly, after exposure to the desired experimental condition, animals were killed. A sternotomy incision was performed and the lungs were removed. The right lower lobe was isolated and immediately weighed (wet weight) before being dried for 48 h at 90 ºC and then weighed again (dry weight).

Statistic analysis. Data were expressed as mean ± SD. Statistical significance was determined by one-way analysis of variance (ANOVA). Significance was subsequently verified with *Student-Newman-Kuels* tests. $P < 0.05$ was considered significant.

Results

Ketamine inhibited endotoxin-induced NF-kappa B activation in the lung

Activity of NF-kappa B in nuclear extracts from the lung with endotoxin stimulation was enhanced significantly when compared with control. Ketamine inhibited NF-kappa B activation at two (5, 50 mg/kg) dosing levels. Ketamine at the dose of 0.5 mg/kg did not inhibit NF-kappa B activation. (Fig. 1)

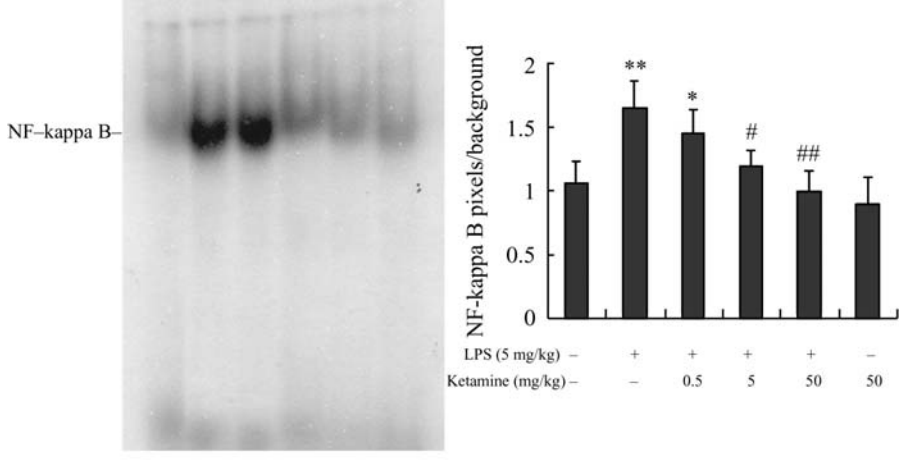

Fig. 1. Effect of ketamine on LPS-induced NF-kappa B activation in the lung. Rats were subjected to saline or endotoxin (5 mg/kg) challenge and treated with or without ketamine (0.5, 5, 50 mg/kg). Two hours later NF-kappa B was investigated in the lung. Endotoxin increased NF-kappa B activation in the lung, and ketamine beyond 5 mg/kg inhibited this response significantly. Lane 1 Control group, lane 2 LPS (5 mg/kg) challenged group, lane 3,4,5 LPS challenged and treated with various dose of ketamine (0.5, 5, 50 mg/kg), lane 6 ketamine alone group (50 mg/kg). The graphic was representative of six. *$p < 0.05$ and **$p < 0.01$ vs control group; #$p < 0.05$ and ##$p < 0.01$ vs LPS only group.

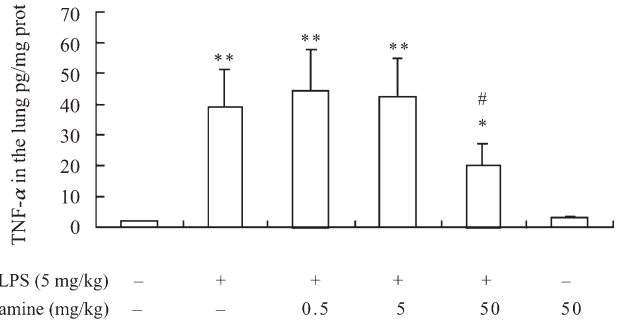

Fig. 2. Effect of ketamine on LPS-induced TNF-α in the lung. Rats were subjected to saline or endotoxin (5 mg/kg) challenge and treated with or without ketamine (0.5, 5, 50 mg/kg). Two hours later TNFα was investigated in the lung. Endotoxin increased TNF-α production in the lung, and ketamine 50 mg/kg inhibited this response significantly. The graphic was representative of six. *p < 0.05 and **p < 0.01 vs control group; #p<0.05 vs LPS only group.

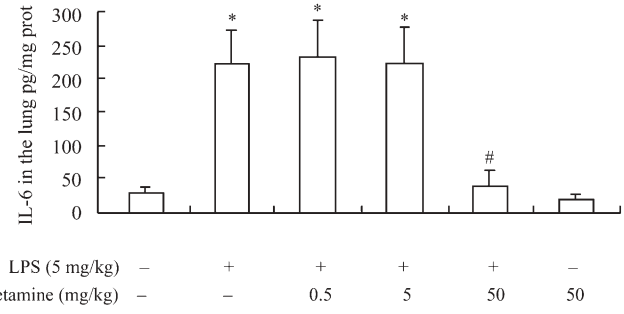

Fig. 3. Effect of ketamine on LPS-induced IL-6 in the lung. Rats were subjected to saline or endotoxin (5 mg/kg) challenge and treated with or without ketamine (0.5, 5, 50 mg/kg). Two hours later IL-6 was investigated in the lung. Endotoxin increased IL-6 production in the lung, and ketamine 50 mg/kg inhibited this response significantly. The graphic was representative of six. *p < 0.05 vs control group; #p < 0.05 vs LPS only group.

Induction of TNF-α and IL-6 in the lung tissue by endotoxin challenge and the protective effect of ketamine

TNF-α and IL-6 remained at baseline level in unchallenged rats. Endotoxin caused an increase in TNF-α and IL-6 in the lung homogenate. Ketamine was administered intraperitoneally soon after the endotoxin challenge and tissue cytokines levels were analyzed 2 h later. Ketamine less than 50 mg/kg could not suppress TNF-α and IL-6 elevation significantly. Ketamine only reaching the dose of 50 mg/kg inhibited TNF-α and IL-6 production in the lung (Fig. 2 and Fig. 3).

Ketamine blunted sepsis-induced lung neutrophil accumulation

We studied the neutrophil influx into the lung tissue using the MPO activity determination. As shown in Table 1, MPO levels were significantly increased from 1.7 ± 0.13 U/g in control animals to 2.57 ± 0.21 U/g in the endotoxin challenged group of rats and to 2.64 ± 0.18 U/g, 2.54 ± 0.2, 2.14 ± 0.11 in the rats treated with both LPS and ketamine (0.5, 5, 50 mg/kg). Control rats and rats treated with ketamine alone had similar lung MPO activities.

Pulmonary Edema Assessment

Intravenous injection of endotoxin into the rats increased wet/dry weight ratio from 4.64 ± 0.32 to 5.65 ± 0.35. They were 5.6 ± 0.29, 5.5 ± 0.2, 5.17 ± 0.30 in groups after endotoxin stimulation and treated with ketamine (0.5, 5, 50 mg/kg). Ketamine alone had no effect on wet/dry weight ratio (Table 1).

Discussion

In our study, we demonstrated that intravenous administration of endotoxin could provoke significant lung injury, which was characterized by neutrophil accumulation, pulmonary edema, tissue cytokines expression and NF-kappa B activation. Ketamine could inhibit these inflammatory responses only at supra-anesthetic dosage.

TNF-α is regarded as the most important proinflammatory cytokine, and is released early after an inflammatory stimulus [15]. IL-6, which increases after TNF-α, contributes to both morbidity and mortality in conditions of "uncontrolled" inflammation [16]. Many effector genes, including those encoding cytokines and adhesion molecules, are in turn regulated by NF-kappa B. NF-kappa B was verified to be the upstream regulator of TNF-α and IL-6. Some anti-inflammatory agents (e. g. salicylates, dexamethasone) can inhibit NF-kappa B, which suggests that it is an important molecular tar-

Table 1. Wet/dry Weight and MPO Activity in the Lung.

Parameter	Control	LPS (5 mg/kg)	LPS+KET (0.5 mg/kg)	LPS+KET (5 mg/kg)	LPS+KET (50 mg/kg)	KET (50 mg/kg)
Wet/dry Weight	4.64 ± 0.32	5.65 ± 0.35[a]	5.6 ± 0.29[a]	5.5 ± 0.26[a]	5.17 ± 0.30[bc]	4.8 ± 0.27
MPO Activity(U/g)	1.7 ± 0.13	2.57 ± 0.21[a]	2.64 ± 0.18[a]	2.54 ± 0.2[a]	2.14 ± 0.11[bc]	1.69 ± 0.14

MPO Activity: Myeloperoxidase Activity; LPS: Lipopolysaccharide; KET: Ketamine.
[a] p < 0.01 vs Control group.
[b] p < 0.05 vs Control group.
[c] p < 0.05 vs LPS only group.

get for the modulation of inflammatory disease [17–18]. In our study, we found ketamine at the dose of 50 mg/kg could decrease neutrophil accumulation into the lung tissue and pulmonary edema from endotoxin challenge, which might act through inhibiting the generation of proinflammatory cytokines and suppressing its gene regulator NF-kappa B. However ketamine at the dose of 5 mg/kg could not inhibit the generation of TNF-α and IL-6 while this dose was found to inhibit NF-kappa B activation, which indicated rather than TNF-α and IL-6, endotoxin-induced NF-kappa B activation was more easily inhibited by ketamine.

The neutrophil is the primary cellular mediator in ARDS, and pulmonary neutrophil accumulation in the patients is evident in lung biopsies, postmortem specimens, and bronchoalveolar lavage fluid. Strategies directed against either neutrophil accumulation or neutrophils function reduce sepsis-induced acute lung injury [19]. In our study, we demonstrated that ketamine decreased neutrophil accumulation into the lung after endotoxin challenge, and this inhibitory effect might act through inhibiting NF-kappa B and some proinflammatory cytokines. Besides, Ketamine could modulate the stimulated adhesion molecule expression on human neutrophils in vitro directly [20]. Therefore ketamine had a multiple regulating effect on endotoxin-induced lung injury.

The dose of ketamine used in our experiment was 0.5–50 mg/kg, which encompasses the clinical range. Previous studies reported that ketamine (> 20 μg/ml) could inhibit endotoxin-induced TNF-α in vitro [6]. Li et al [21] showed that more than 2.7 μg/ml ketamine inhibited nitric oxide production in LPS-treated rat alveolar macrophages. The concentration of ketamine in human plasma could reach 30 μg/ml by the intravenous administration of ketamine 2–2.2 mg/kg [22]. Roytblat et al. reported that a single dose of ketamine 0.25 mg/kg administered before cardiopulmonary bypass suppressed the increase in serum IL-6 during and after coronary artery bypass surgery [23]. Although others have failed to demonstrate any effect at that dose [24–25]. In our in vivo study, ketamine suppressed endotoxin-induced pulmonary inflammation only at the dose of 50 mg/kg, which indicated that sub-anesthetic or anesthetic dose of ketamine might have not anti-inflammatory effect in rats in vivo. However we could not exclude there were differences between humans and animals and/or between in vitro and in vivo studies. Since large concentration of ketamine might have nonspecific cytostatic effect [26], we had to hypothesize that administration of 50mg/kg ketamine in vivo might have nonspecific cytostatic effect to the body.

Therefore, we conclude that ketamine at a sub-anesthetic or anesthetic dosage could not inhibit endotoxin-induced pulmonary inflammation in vivo. Supra-anesthetic dose of ketamine might have anti-inflammatory effect. However we do not advocate to use large dose of ketamine to deal with sepsis.

References

[1] Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med 2000; 342: 1334–49.

[2] Zilberberg MD, Epstein SK. Acute lung injury in the medical ICU: co-morbid conditions, age, etiology, and hospital outcome. Am J Respir Crit Care Med 1998; 157: 1159–64.

[3] Abel SJ, Finney SJ, Brett SJ, Keogh BF, Morgan CJ, Evans TW. Reduced mortality in association with the acute respiratory distress syndrome (ARDS).Thorax 1998; 53: 292–4.

[4] Hudson LD, Milberg JA, Anardi D, Maunder RJ. Clinical risks for development of the acute respiratory distress syndrome. Am J Respir Crit Care Med 1995; 151: 293–301.

[5] Yli-Hankala A, Kirvela M, Randell T, Lindgren L. Ketamine anaesthsia in a patient with septic shock. Acta Anaesthsiol Scand 1992; 36: 483–5.

[6] Kawasaki T, Ogata M, Kawasaki C, Ogata J, Inoue Y, Shigematsu A. Ketamine Suppresses Proinflammatory Cytokine Production in Human Whole Blood In Vitro. Anesth Analg 1999; 89: 665–9.

[7] Yu Y, Zhou Z, Xu J, Liu Z, Wang Y. Ketamine reduces NF-kappa B activation and TNF alpha production in rat mononuclear cells induced by lipopolysaccharide in vitro. Ann Clin Lab Sci 2002; 32: 292–8.

[8] Wischmeyer PE, Kahana M, Wolfson R, Ren H, Musch MM, Chang EB. Glutamine reduces cytokine release, organ damage, and mortality in a rat model of endotoxemia. Shock 2001; 16: 398–402.

[9] Murakami K, Okajima K, Uchiba M. The prevention of lipopolysaccharide-induced pulmonary vascular injury by pretreatment with cepharanthine in rats. Am J Respir Crit Care Med 2000; 161: 57–63.

[10] Liu Z**,** Yu Y, Jiang Y, Li J. Growth hormone increases lung NF-kappa B activation and lung microvascular injury induced by lipopolysaccharide in rats. Ann Clin Lab Sci 2002; 32: 164–70.

[11] Zhou W, Jiang ZW, Tian J, Jiang J, Li N, Li JS. Role of NF-κB and cytokine in experimental cancer cachexia. World J Gastroenterol 2003; 9: 1567–70.

[12] Sun J, Li F, Chen J, Xu J. Effect of Ketamine on NF-kappa B Activity and TNF-alpha Production in Endotoxin-Treated Rats. Ann Clin Lab Sci 2004; 34: 181–6.

[13] Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. Gastroenterology 1984; 87: 1344–50.

[14] Nielsen VG, Sidharta T, Weinbroum A, McCammon AT, Samuelson PN, Gelman S et al. Lung injury after hepatoenteric ischemia-reperfusion: role of xanthine oxidase. Am J Respir Crit Care Med 1996; 154: 1364–9.

[15] Hesse DG., Tracey KJ, Fong Y, Manogue KR, Palladino MA, Cerami A et al. Cytokine appearance in human endotoxemia and primate bacteremia. Surg Gynecol Obstet 1988; 166: 147–53.

[16] Damas P, Ledoux D, Nys M, Vrindts Y, Groote D, Franchimont P et al. Cytokine serum level during severe sepsis in human: IL-6 as a marker of severity. Ann Surg 1992; 215: 356–62.

[17] Amann R, Peskar BA. Anti-inflammatory effects of aspirin and sodium salicylate. Eur J Pharmacol 2002; 447: 1–9.

[18] Crinelli R, Antonelli A, Bianchi M, Gentilini L, Scaramucci S, Magnani M. Selective inhibition of NF-κB activation and TNF-alpha production in macrophages by red blood cell-mediated delivery of dexamethasone. Blood Cells Mol Dis 2000; 26: 211–22.

[19] Pulido EJ, Shames BD, Pennica D, O'Leary RM, Bensard DD, Cain BS et al. Cardiotrophin-1 Attenuates Endotoxin-Induced Acute Lung Injury. J Surg Res 1999; 84: 240–6.

[20] 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.

[21] 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.

[22] Domino EF, Zsigmond EK, Domino LE, Domino KE, Kothary SP, Domino SE. Plasma levels of ketamine and two of its metabolites in surgical patients using a gas chromatographic mass fragmentographic assay. Anesth Analg 1982; 61: 87–92.

[23] Roytblat L, Talmor D, Rachinsky M, Greemberg L, Pekar A, Appelbaum A et al. Ketamine attenuates the interleukin-6

response after cardiopulmonary bypass. Anesth Analg 1998; 87: 266–71.
[24] Takaono M, Yogosawa T, Okawa-Takatsuji M, Aotsuka S. Effects of intravenous anesthetics on IL-6 and IL-10 production by lipopolysaccharide-stimulated mononuclear cells from healthy volunteers. Acta Anaesthesiol Scand 2002; 46: 176–9.
[25] Hoff G, Bauer I, Larsen B, Bauer M. Modulation of endotoxin-stimulated TNF-alpha gene expression by ketamine and propofol in cultured human whole blood. Anaesthesist 2001; 50: 494–9.
[26] Lewis E, Rogachev B, Shaked G, Douvdevani A. The in vitro effects of ketamine at large concentrations can be attributed to a nonspecific cytostatic effect. Anesth Analg 2001; 92: 927–9.