Effect of isoflurane + N_2O inhalation and propofol + fentanyl anesthesia on myocardial function as assessed by cardiac troponin, caspase-3, cyclooxygenase-2 and inducible nitric oxide synthase expression

ZHUANGHUI ZHU[1*], SHUANGLIAN YI[2*], ZHONGGUI SHAN[1,3], HONGWEI GUO[1] and SHAOFAN KE[1]

[1]Department of Cardiac Surgery, The First Affiliated Hospital of Xiamen University; [2]Department of Gastroenterology, Zhongshan Hospital of Xiamen University; [3]Department of Cardiac Surgery, Teaching Hospital of Fujian Medical University, Xiamen, Fujian 361003, P.R. China

Received February 21, 2016; Accepted May 11, 2017

DOI: 10.3892/etm.2017.5029

Abstract. The aim of this study was to evaluate the effect of isoflurane + N_2O inhalation and propofol + fentanyl anesthesia on myocardial function as assessed by cardiac troponin T (cTnT). A total of 60 patients were randomized into two groups: isoflurane + N_2O inhalation (n=30) and propofol + fentanyl anesthesia (n=30). The findings demonstrated that there was no significant difference between the two experimental groups in terms of cTnT levels, demographic properties or hemodynamic parameters. Isoflurane + N_2O inhalation and propofol + fentanyl anesthesia, respectively, were also investigated in a rat model of myocardial infarction. Myocardial cell damage, inflammation and oxidative stress levels, caspase-3/9 activities and cyclooxygenase-2 protein expression were markedly decreased, although there was no statistical significance difference between the two experimental groups. Notably, inducible nitric oxide synthase protein expression in the isoflurane + N_2O inhalation group was significantly higher than that of the propofol + fentanyl anesthesia group ($P<0.01$). In conclusion, isoflurane + N_2O inhalation and propofol + fentanyl anesthesia are not associated with risks for myocardial function.

Introduction

Myocardial infarction (MI), commonly referred to as a heart attack, occurs when blood flow stops to part of the heart causing damage to the heart muscle (1). With increasing morbidity and death rates, it has become a great threat to human health. The leading cause of MI at the acute phase is acute heart failure (2). With the development of medical techniques, thrombolysis, interventional stent and bypass surgery have greatly decreased mortality rates of the conversion from acute MI to acute heart failure (3). However, heart failure can be become chronic. Priority is given to remodeling of cardiac fibrosis for chronic MI. Excessive cardiac fibrosis remodeling causes heart failure.

As an inhalation anesthetic, isoflurane is does not irritate the respiratory system (4). When used as an anesthesia for teenagers, adults and the elderly, it is stable in induction with high recovery quality (5). N_2O is not toxic, has strong analgesic effects and patients are quickly awoken (5). Consequently, it is widely employed in clinics.

With high lipid solubility and low water solubility, blood concentrations of N_2O peak after intravenous injection of 2.5 mg/kg of propofol after 2 min (6). Redistribution quickly occurs, resulting in a quick decrease in blood concentration (5). Characterized by quickly taking effect without drug accumulation and patients being easily awoken, propofol metabolizes in the liver and its elimination half life is 30-60 min (7). Fentanyl is a newly discovered narcotic opiate analgesic, which has similar pharmacological functions to other opiates, such as relieving pain and calming (8). The efficacy of fentanyl peaks 3-5 min after intravenous injection and metabolizes through the liver. Its terminal half time is 2 to 4 h (9). With specific pharmacokinetic features, it takes effect fast and peaks after 1.6 min of intravenous injection. It can be degraded via non-specific esterase in red blood cells and tissues (10). With a terminal half life of 0.1 to 0.6 h, its clearance rates are not influenced by hepatic and renal functions, which is a key advantage in addition to its high safety index, short waking time, reduced respiratory depression and stable hemodynamics (11).

In the present study, the effect of isoflurane + N_2O inhalation and propofol + fentanyl anesthesia on myocardial function was investigated, as assessed by cardiac troponin in patients or the established rat model.

Correspondence to: Mr. Zhonggui Shan, Department of Cardiac Surgery, The First Affiliated Hospital of Xiamen University, 55 Zhenhai Road, Xiamen, Fujian 361003, P.R. China
E-mail: zhongguishanss@163.com

*Contributed equally

Key words: isoflurane + N_2O inhalation, propofol + fentanyl anesthesia, myocardial function, inducible nitric oxide synthase, cyclooxygenase-2

Materials and methods

Ethics statement. This study was approved by the Ethics Committee (grant no. 20081009) of Zhongshan Hospital affiliated to Xiamen University (Xiamen, China). Written informed consent was obtained from all participants who were involved in the study. All procedures involving experimental animals were performed in accordance with the protocols that were approved by the Committee for Animal Research of Xiamen University and complied with the Guideline for the Care and Use of Laboratory Animals.

Study population and administration of anesthesia. All patients were aged 20-45 years and had been admitted to The First Affiliated Hospital of Xiamen University between July 2013 and December 2014. Patients were randomly split into two equal groups. The following inclusion criteria was applied to all patients in the present study: i) Patients with ongoing myocardial damage (LV dysfunction, electrocardiographic abnormalities or elevated troponin); and ii) patients exhibtied new onset or persistent symptoms suggestive of myocarditis. The following exclusion criteria was followed: i) exclusion for history of systemic viral disease; and ii) exclusion for relevant coronary artery disease (CAD) on angiography. One group received isoflurane + N_2O inhalation (n=30) and the other group received propofol + fentanyl anesthesia (n=30). In the isoflurane + N_2O inhalation group, patients were given 1.5% isoflurane + NO_2/O_2 (50/50%) after induction with thiopental sodium (5 mg/kg) + fentanyl (1 μg/kg). In the propofol + fentanyl anesthesia group, patients were given propofol (0.2 mg/kg/min) and fentanyl (0.2 μg/kg/min), and ventilated with 50% O_2 and 50% air, after induction with propofol (3 mg/kg) and fentanyl (5 μg/kg).

Animals and experimental procedures. A total of 20 male Sprague-Dawley rats (aged 8 weeks) were housed at 22-24˚C, 12-h light/dark cycle and 50-60% humidity with free access to food and water. All Sprague-Dawley rats were randomly assigned into two groups: One group received isoflurane + N_2O inhalation (n=10) and the other group received propofol + fentanyl anesthesia (n=10). In the isoflurane + N_2O inhalation group, rats were performed with 1.5% isoflurane + NO_2/O_2 (50%/50%) in the buffer for 30 min after induction with thiopental sodium (5 mg/kg) + fentanyl (1 μ/kg). In the propofol + fentanyl anesthesia group, rats were administered propofol (0.2 mg/kg/min) and fentanyl (0.2 μg/kg/min), and ventilated with 50% O_2 and 50% air in the buffer for 30 min after induction with propofol (3 mg/kg) and fentanyl (5 μg/kg).

To establish MI in rats, all the rats were anesthetized with 35 mg/kg of pentobarbital and carefully catheterized with a stump needle. An incision was cut along the left side of sternum, the thorax was opened and the pericardium was exposed. Left coronary artery (LCA) was ligated via using a 6-0 prolene suture and the chest was closed.

Hematoxylin and eosin (H&E) staining. Hearts were harvested from the experiment rats and perfusion-fixed with 4% buffered formalin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 24 h at room temperature. Tissue samples were horizontally sectioned and embedded in paraffin. Tissue sections were cut into 5-μm thick slices and subsequently stained with H&E (Beyotime Institute of Biotechnology, Shanghai, China) for 5-10 min at room temperature and analyzed using Image Pro-Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA).

Detection index. Blood samples (500 μl) were obtained from rats after treatment with isoflurane + N_2O inhalation and propofol + fentanyl anesthesia, and transferred to sterile tubes without EDTA and heparin prior to centrifugation at 3,000 x g for 10 min at 4˚C, and serum was collected and storage at -20˚C. Nuclear factor (NF)-κB of p65 (ml003404), interleukin (IL)-6 (ml102828), superoxide dismutase (SOD; ml540172), glutathione (GSH; ml531010), glutathione peroxidase (GSH-PX; ml097316) and malondialdehyde (MDA; ml022446) activities were measured using ELISA kits (Shanghai Enzyme-linked Biotechnology Co, Shanghai, China). Caspase-3 and -9 activity was measured using Ac-DEVD-pNA/LEHD-pNA (C1115 and C1157; Beyotime Institute of Biotechnology; Nanjing, China) and incubated for 2 h at 37˚C. Activities were determined using an ELISA plate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Western blot analysis. Total protein from rat hippocampi was extracted using protein lysis buffer containing protease inhibitor (PMSF; Beyotime Institute of Biotechnology; Nanjing, China) at 4˚C for 30 min and centrifuged for 10 min at 4˚C at 12,000 x g. Total cellular proteins (50 μg) were separated by 12% SDS-PAGE and electrotransferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories, Inc.). The membrane was blocked with 5% skimmed milk powder in Tris-buffered saline with Tween-20 (TBST) for 1 h at 37˚C and incubated with the following primary antibodies: Anti-cyclooxygenase-2 (sc-7951; COX-2; 1:3,000), anti-inducible nitric oxide synthase (sc-649; iNOS; 1:3,000) and anti-β-actin (sc-7210; 1:5,000; all Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at 4˚C overnight. Subsequently, the membrane was washed with TBST three times for 15 min and incubated with an anti-mouse or anti-rabbit IgG secondary antibody conjugated to horseradish peroxidase (sc-2004, 1:5,000; Santa Cruz Biotechnology, Inc.) for 1 h at 37˚C and visualized using enhanced chemiluminescence reagent (Beyotime Institute of Biotechnology, Jiangsu, China). Protein bands were analyzed using Bio-Rad Laboratories Quantity One software 3.0 (Bio-Rad Laboratories, Inc.).

Statistical analysis. Data are presented as the mean ± standard deviation and were analyzed using SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL, USA). One-way analysis of variance tests were performed followed by the Student-Newman-Keuls test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Basic characteristics of patients. In the isoflurane + N_2O inhalation group, the mean age of patients was 37.81±6.71 years, their body weight was 65.59±2.12 kg and the female:male ratio was 20:0 (Table I). In the propofol + fentanyl anesthesia group, the mean age of patients was 38.33±6.21 years, their body

Table I. Basic characteristics of the patients in the two groups.

Group	Control group	Intervention group	P-value
Age (years)	37.81±6.71	38.33±6.21	0.812
Body weight (kg)	65.59±2.12	66.48±1.89	0.871
Sex (F/M)	20/0	20/0	

Data are presented as the mean ± standard deviation or F/M, as indicated. F, female; M, male.

Table II. Hemodynamic parameters of the patients in the two groups.

Parameters	Control group	Intervention group	P-value
Before induction			
SBP (mm/Hg)	126.28±4.71	125.91±5.21	0.871
DBP (mm/Hg)	76.12±2.33	76.72±2.67	0.912
Pulse (per min)	80.91±2.47	79.87±2.03	0.638
O_2 saturation (%)	96.14±0.51	96.21±0.57	0.899
After induction			
SBP (mm/Hg)	127.58±5.26	132.91±4.94	0.661
DBP (mm/Hg)	83.07±1.93	79.67±2.38	0.697
Pulse (per min)	89.51±3.08	76.92±3.18	0.001
O_2 saturation (%)	97.93±0.39	97.01±0.49	0.812

Data are presented as the mean ± standard deviation. SBP, systolic blood pressures; DBP, diastolic blood pressure.

weight was 66.48±1.89 kg and the female:male ratio was 20:0 (Table I).

Hemodynamic parameters in patients. Prior to induction, as shown in Table II, the preanesthetic and anesthetic hemodynamic parameters of patients in the isoflurane + N_2O inhalation group were similar to those of patients in the propofol + fentanyl anesthesia group (P>0.05). However, the pulse rates of those in the isoflurane + N_2O inhalation group were lower than that of the propofol + fentanyl anesthesia group (P<0.05; Table II).

Cardiac troponin T (cTnT) levels in patients. Levels of cTnT and troponin from the two patient groups are presented in Tables III and IV. There was no significant inter-group difference between the isoflurane + N_2O inhalation group and propofol + fentanyl anesthesia group when assessing the level of cTnT and troponin (P>0.05; Tables III and IV).

H&E staining in rat models of myocardial infarction (MI). To examine the effect of isoflurane + N_2O inhalation and propofol + fentanyl anesthesia on myocardial function in rats, a model of MI was induced using male Sprague-Dawley rats. As shown in Fig. 1, there was no notable difference between these two experimental groups in terms of myocardial cell damage.

Table III. Cardiac troponin T levels of the patients in the two groups.

Variable (ng/ml)	Control group	Intervention group	P-value
Preanesthetic	0.22±0.02	0.23±0.03	0.276
Anesthetic	0.24±0.03	0.21±0.01	0.179
Postanesthetic	0.23±0.03	0.22±0.03	0.233

Data are presented as the mean ± standard deviation.

Table IV. Troponin levels of the patients in the two groups.

Group	Control group (P-value)	Intervention group (P-value)
Preanesthetic/anesthetic	0.731	0.392
Anesthetic/postanesthetic	0.512	0.478

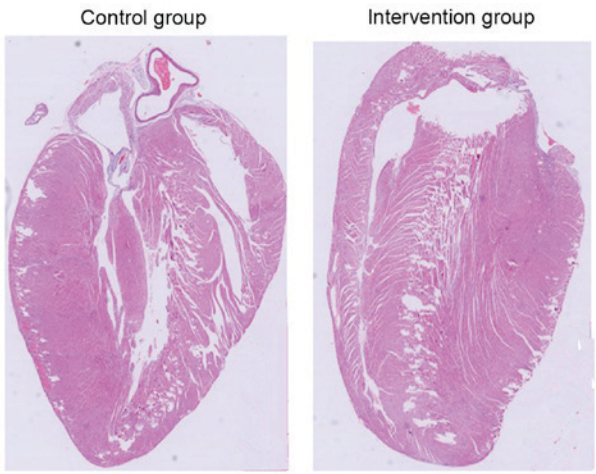

Figure 1. Hematoxylin and eosin staining of heart samples from rat models of myocardial infarction. The control group received propofol + fentanyl anesthesia, whereas the intervention group were administered isoflurane + N_2O inhalation.

Inflammation levels in rat models of MI. To examine whether the effect of isoflurane + N_2O inhalation and propofol + fentanyl anesthesia on inflammation levels in rats, NF-κB of p65 and IL-6 activities were measured. As shown in Fig. 2, NF-κB of p65 and IL-6 activities of isoflurane + N_2O inhalation group were significantly lower than those of the propofol + fentanyl anesthesia group (P<0.05). These findings demonstrated that propofol + fentanyl anesthesia possesses anti-inflammation effects in rats.

Oxidative stress levels in rat models of MI. To further investigate the effect of isoflurane + N_2O inhalation and propofol + fentanyl

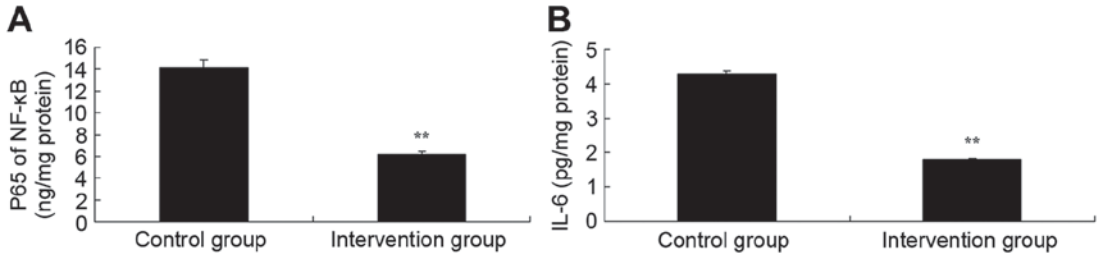

Figure 2. Inflammation levels in rat models of myocardial infarction. Activities of (A) NF-κB of p65 and (B) IL-6. The control group received propofol + fentanyl anesthesia, whereas the intervention group were administered isoflurane + N_2O inhalation. **P<0.01 vs. the control group. NF-κB of p65, nuclear factor-κB of p65; IL-6, interleukin-6.

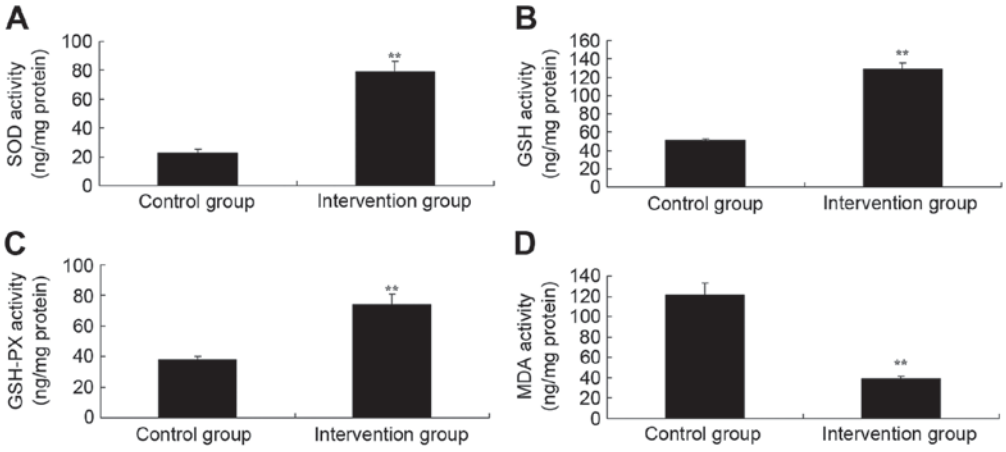

Figure 3. Oxidative stress levels in rat models of myocardial infarction. Activities of (A) SOD, (B) GSH, (C) GSH-PX and (D) MDA. The control group received propofol + fentanyl anesthesia, whereas the intervention group were administered isoflurane + N_2O inhalation. **P<0.01 vs. the control group. SOD, superoxide dismutase; GSH, glutathione; GSH-PX, glutathione peroxidase; MDA, malondialdehyde.

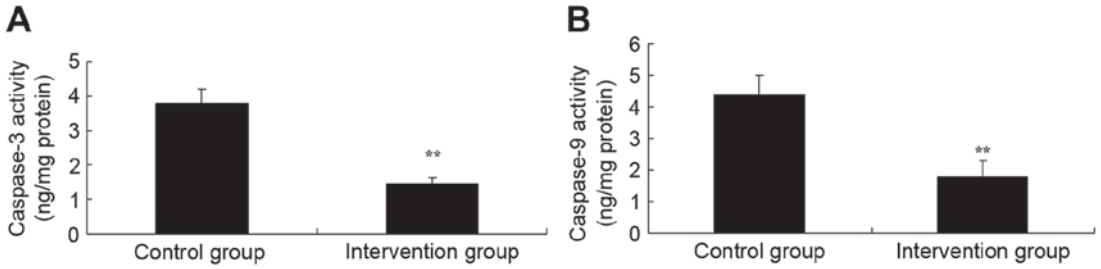

Figure 4. (A) Caspase-3 and (B) caspase-9 activity in rat models of myocardial infarction. The control group received propofol + fentanyl anesthesia, whereas the intervention group were administered isoflurane + N_2O inhalation. **P<0.01 vs. the control group.

anesthesia on oxidative stress levels in rats, the activities of SOD, GSH, GSH-PX and MDA were measured using ELISA kits. As shown in Fig. 3, the activities of SOD, GSH and GSH-PX of isoflurane + N_2O inhalation group were lower and those of propofol + fentanyl anesthesia group; whereas the activity of MDA in the isoflurane + N_2O inhalation group was significantly lower than that of the propofol + fentanyl anesthesia group. These findings demonstrated that propofol + fentanyl anesthesia also possesses anti-oxidative effects in rats.

Caspase-3 and -9 activity. Using ELISA kits, the effects of isoflurane + N_2O inhalation and propofol + fentanyl anesthesia on caspase-3 and -9 activity were determined in the rat models of MI. As shown in Fig. 4, caspase-3 and -9 activities in the isoflurane + N_2O inhalation group were significantly suppressed, as compared with the propofol + fentanyl anesthesia group (P<0.05). The study indicated that propofol + fentanyl anesthesia inhibited caspase-3/9 activity to reduce heart cell apoptosis in in rats of MI.

COX-2 protein expression. Using western blot analysis, the effect of isoflurane + v inhalation and propofol + fentanyl anesthesia on COX-2 protein expression levels was determined. Western blot analysis demonstrated that COX-2 protein expression levels in the isoflurane + N_2O inhalation group were significantly inhibited, as compared with the propofol + fentanyl anesthesia group, which suggested that propofol + fentanyl anesthesia weakened COX-2 protein

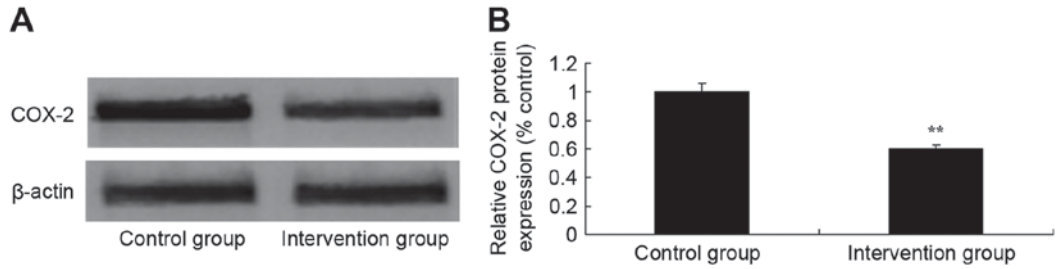

Figure 5. COX-2 protein expression in rat models of myocardial infarction. COX-2 protein expression levels were assessed via (A) western blotting and (B) quantified. The control group received propofol + fentanyl anesthesia, whereas the intervention group were administered isoflurane + N_2O inhalation. **P<0.01 vs. the control group. COX-2, cyclooxygenase-2.

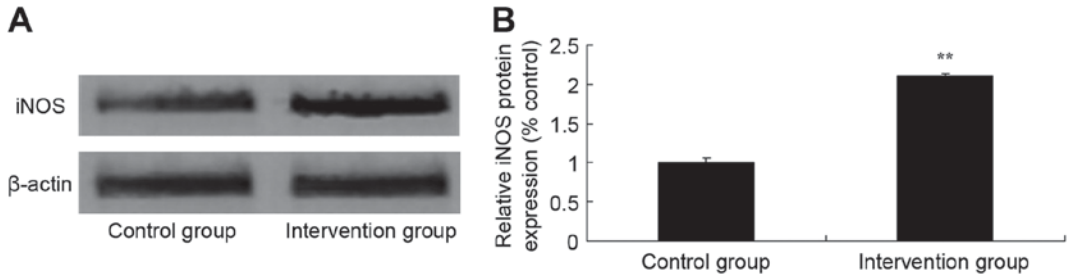

Figure 6. iNOS protein expression in rat models of myocardial infarction. iNOS protein expression levels were assessed via (A) western blotting and (B) quantified. The control group received propofol + fentanyl anesthesia, whereas the intervention group were administered isoflurane + N_2O inhalation. **P<0.01 vs. the control group. iNOS, inducible nitric oxide synthase.

expression and prevented inflammation in rats of MI (P<0.05; Fig. 5).

iNOS protein expression. To further confirm the effect of isoflurane + N_2O inhalation and propofol + fentanyl anesthesia on iNOS protein expression, iNOS protein expression levels were detected using western blot analysis. As shown in Fig. 6, iNOS protein expression in the isoflurane + N_2O inhalation group was significantly higher than that of the propofol + fentanyl anesthesia group (P<0.05). These findings demonstrated that propofol + fentanyl anesthesia also promoted iNOS protein expression in rats of MI.

Discussion

MI is an ischemic heart disease. When the coronary artery or branches have lesions, stenosis or blocking occurs, which causes myocardial ischemic-anoxic injury or necrosis (12). Neither traditional therapy nor newer intervention therapy has been able to achieve satisfactory effects for diffuse injuries of coronary arterioles (13). This is mainly as blood micro-circulation at the ischemic region cannot be improved.

In this study, the pulse rates of the isoflurane + N_2O inhalation group were lower than that of the propofol + fentanyl anesthesia group. Clinically, the judgment of anesthesia depth for patients is based on heart rate, blood pressure and body movement (14). However, the dose of propofol was decreased in anesthetic depth monitoring, which suggests that blood pressure and heart rate are inadequate for judging anesthesia depth, which may lead to excessive dosages of anesthetic drugs (6). Our study showed that no significant inter-group difference existed between the isoflurane + N_2O inhalation group and the propofol + fentanyl anesthesia group in terms of the levels of cTnT and troponin.

N_2O, which is colorless, odorless and non-irritating, is an inorganic gas with a low blood/gas distribution coefficient (5). In addition to rapid induction, quick waking and non-irritation of the respiratory tract, N_2O does not damage important organs, such as the heart, lung, liver and kidneys (15). It does not participate in bio-conversion or degradation *in vivo*, thus it is predominantly discharged when breathing (16). There is minimal evaporation through skin without accumulation; therefore, N_2O is an ideal inhaled anesthetic (17). As anesthesia performance of $N_2O$is low and weak, a high concentration of single use may cause anoxia; thus, its concentration should not be higher than 60% (18). We found that NF-κB of p65 and IL-6, caspase-3 and 9 activities in the isoflurane + N_2O inhalation group were suppressed, compared with the propofol + fentanyl anesthesia group.

Fentanyl-propofol is a common intravenous anesthesia (19). However, respiratory depression of fentanyl during surgery is rather common and hyperpathia after surgery occurs frequently (19). Propofol is an alkylphenolsedative-hypnotic drug and its advantages include quick effects, short hold time and fast waking (20). It is eliminated through the liver without accumulation. It also has weak antiemetic and analgesic functions (21). The greatest disadvantage of propofol is dose-dependent respiratory inhibition and the fact that it is influenced by injection rates (22). Large dosages induce notable decreases in blood pressure, heart rate, hyoxemia, bradypnea or apnea. With short-term effect, fentanyl is a new opium analgesics (23). It can be degraded quickly through non-specific esterase in blood and tissues and its elimination rate is not affected by hepatorenal functions (24). Characterized by taking effect quickly, strong

abirritation, quick recovery times and a lack of accumulation, it rarely exhibits side effects, such as decreased blood pressure, declined heart rate and breath inhibition, which are related with dosage and injection rates (25). COX-2 and iNOS protein expression levels in the isoflurane + N_2O inhalation group were inhibited and activated, respectively, as compared with the propofol + fentanyl anesthesia group. In conclusion, isoflurane + N_2O inhalation and propofol + fentanyl anesthesia were demonstrated to not affect myocardial function.

References

1. Bayir H, Kapralov AA, Jiang J, Huang Z, Tyurina YY, Tyurin VA, Zhao Q, Belikova NA, Vlasova II, Maeda A, *et al*: Peroxidase mechanism of lipid-dependent cross-linking of synuclein with cytochrome C: Protection against apoptosis versus delayed oxidative stress in Parkinson disease. J Biol Chem 284: 15951-15969, 2009.
2. Huerta C, Sánchez-Ferrero E, Coto E, Blázquez M, Ribacoba R, Guisasola LM, Salvador C and Alvarez V: No association between Parkinson's disease and three polymorphisms in the eNOS, nNOS, and iNOS genes. Neurosci Lett 413: 202-205, 2007.
3. Przedborski S, Jackson-Lewis V, Yokoyama R, Shibata T, Dawson VL and Dawson TM: Role of neuronal nitric oxide in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurotoxicity. Proc Natl Acad Sci USA 93: 4565-4571, 1996.
4. Pontone GM, Palanci J, Williams JR and Bassett SS: Screening for DSM-IV-TR cognitive disorder NOS in Parkinson's disease using the Mattis Dementia Rating Scale. Int J Geriatr Psychiatry 28: 364-371, 2013.
5. Manosroi A, Kitdamrongtham W, Ishii K, Shinozaki T, Tachi Y, Takagi M, Ebina K, Zhang J, Manosroi J, Akihisa R and Akihisa T: Limonoids from *Azadirachta indica* var. *Siamensis* extracts and their cytotoxic and melanogenesis-inhibitory activities. Chem Biodivers 11: 505-531, 2014.
6. Martins VL, Caley MP, Moore K, Szentpetery Z, Marsh ST, Murrell DF, Kim MH, Avari M, McGrath JA, Cerio R, *et al*: Suppression of TGFβ and Angiogenesis by Type VII Collagen in Cutaneous SCC. J Natl Cancer Inst 108: pii: djv293, 2015.
7. Lindholm EE, Aune E, Noren CB, Seljeflot I, Hayes T, Otterstad JE and Kirkeboen KA: The anesthesia in abdominal aortic surgery (ABSENT) study: A prospective, randomized, controlled trial comparing troponin T release with fentanyl-sevoflurane and propofol-remifentanil anesthesia in major vascular surgery. Anesthesiology 119: 802-812, 2013.
8. Klein BY, Tamir H, Hirschberg DL, Ludwig RJ, Glickstein SB, Myers MM and Welch MG: Oxytocin opposes effects of bacterial endotoxin on ER-stress signaling in Caco2BB gut cells. Biochim Biophys Acta 1860: 402-411, 2016.
9. Yoneshima E, Okamoto K, Sakai E, Nishishita K, Yoshida N and Tsukuba T: The transcription factor EB (TFEB) regulates osteoblast differentiation through ATF4/CHOP-dependent pathway. J Cell Physiol 23: 1321-1333, 2016.
10. Restuccia A, Tian YF, Collier JH and Hudalla GA: Self-assembled glycopeptide nanofibers as modulators of galectin-1 bioactivity. Cell Mol Bioeng 8: 471-487, 2015.
11. Deng LJ, Peng QL, Wang LH, Xu J, Liu JS, Li YJ, Zhuo ZJ, Bai LL, Hu LP, Chen WM, *et al*: Arenobufagin intercalates with DNA leading to G2 cell cycle arrest via ATM/ATR pathway. Oncotarget 6: 34258-34275, 2015.
12. Shrivastava P, Vaibhav K, Tabassum R, Khan A, Ishrat T, Khan MM, Ahmad A, Islam F, Safhi MM and Islam F: Anti-apoptotic and anti-inflammatory effect of Piperine on 6-OHDA induced Parkinson's rat model. J Nutr Biochem 24: 680-687, 2013.
13. Yasuda T, Hayakawa H, Nihira T, Ren YR, Nakata Y, Nagai M, Hattori N, Miyake K, Takada M, Shimada T, *et al*: Parkin-mediated protection of dopaminergic neurons in a chronic MPTP-minipump mouse model of Parkinson disease. J Neuropathol Exp Neurol 70: 686-697, 2011.
14. Zhao Y, Wang D, Xu T, Liu P, Cao Y, Wang Y, Yang X, Xu X, Wang X and Niu H: Bladder cancer cells re-educate TAMs through lactate shuttling in the microfluidic cancer microenvironment. Oncotarget 6: 39196-39210, 2015.
15. Soares DG, Godin AM, Menezes RR, Nogueira RD, Brito AM, Melo IS, Coura GM, Souza DG, Amaral FA, Paulino TP, *et al*: Anti-inflammatory and antinociceptive activities of azadirachtin in mice. Planta Med 80: 630-636, 2014.
16. Kim WH, Song HO, Jin CM, Hur JM, Lee HS, Jin HY, Kim SY and Park H: The methanol extract of *Azadirachta indica* A. Juss leaf protects mice against lethal endotoxemia and sepsis. Biomol Ther (Seoul) 20: 96-103, 2012.
17. Omobowale TO, Oyagbemi AA, Oyewunmi OA and Adejumobi OA: Chemopreventive effect of methanolic extract of *Azadirachta indica* on experimental *Trypanosoma brucei* induced oxidative stress in dogs. Pharmacognosy Res 7: 249-258, 2015.
18. Dkhil MA, Al-Quraishy S, Abdel Moneim AE and Delic D: Protective effect of *Azadirachta indica* extract against *Eimeria papillata*-induced coccidiosis. Parasitol Res 112: 101-106, 2013.
19. Rodriguez DAO, de Lima RF, Campos MS, Costa JR, Biancardi MF, Marques MR, Taboga SR and Santos FCA: Intrauterine exposure to bisphenol A promotes different effects in both neonatal and adult prostate of male and female gerbils (*Meriones unguiculatus*). Environ Toxicol 31: 1740-1750, 2016.
20. Yue K, Trujillo-de Santiago G, Alvarez MM, Tamayol A, Annabi N and Khademhosseini A: Synthesis, properties and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. Biomaterials 73: 254-271, 2015.
21. Yan Y, Martin LM, Bosco DB, Bundy JL, Nowakowski RS, Sang QX and Li Y: Differential effects of acellular embryonic matrices on pluripotent stem cell expansion and neural differentiation. Biomaterials 73: 231-242, 2015.
22. Koyano-Nakagawa N, Shi X, Rasmussen TL, Das S, Walter CA and Garry DJ: Feedback mechanisms regulate Ets variant 2 (Etv2) gene expression and hematoendothelial lineages. J Biol Chem 290: 28107-28119, 2015.
23. Warsinske HC, Ashley SL, Linderman JJ, Moore BB and Kirschner DE: Identifying mechanisms of homeostatic signaling in fibroblast differentiation. Bull Math Biol 77: 1556-1582, 2015.
24. Miyamoto DT, Zheng Y, Wittner BS, Lee RJ, Zhu H, Broderick KT, Desai R, Fox DB, Brannigan BW, Trautwein J, *et al*: RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. Science 349: 1351-1356, 2015.
25. Khoo NK, Cantu-Medellin N, St Croix C and Kelley EE: In vivo immuno-spin trapping: Imaging the footprints of oxidative stress. Curr Protoc Cytom 74: 12.42.1-11, 2015.