Effects of nitrous oxide on the production of cytokines and chemokines by the airway epithelium during anesthesia with sevoflurane and propofol

SEIICHIRO KUMAKURA[1], KEISUKE YAMAGUCHI[1], YUSUKE SUGASAWA[1], TAISUKE MURAKAMI[2], TOSHIHIRO KIKUCHI[1], EIICHI INADA[3] and ISAO NAGAOKA[2]

[1]Department of Anesthesiology and Pain Medicine, Juntendo University Nerima Hospital, Tokyo 177-8521; [2]Department of Host Defense and Biochemical Research, Juntendo University Graduate School of Medicine; [3]Department of Anesthesiology and Pain Medicine, Juntendo University School of Medicine, Tokyo 113-8431, Japan

Received April 22, 2013; Accepted October 11, 2013

DOI: 10.3892/mmr.2013.1745

Abstract. The aim of this study was to evaluate the effects of nitrous oxide (a gaseous anesthetic) on the *in vivo* production of inflammatory cytokines and chemokines by the airway epithelium, when combined with sevoflurane or propofol. Subjects undergoing simple or segmental mastectomy were randomly assigned to the sevoflurane and nitrous oxide, sevoflurane and air, propofol and nitrous oxide, or propofol and air group (all n=13). Epithelial lining fluid (ELF) was obtained using the bronchoscopic microsampling method prior to and following the mastectomy to enable measurement of the pre- and post-operative levels of certain inflammatory cytokines and chemokines using a cytometric bead array system. Notably, the levels of interleukin (IL)-1β, IL-8 and monocyte chemotactic protein-1 (MCP-1) in the ELF were significantly increased following the operations which involved the inhalation of sevoflurane and nitrous oxide, although the levels of these molecules were not significantly changed by the inhalation of sevoflurane and air. Furthermore, the IL-12p70 levels were significantly reduced in the ELF following the operations that involved the inhalation of sevoflurane and air, although the IL-12p70 levels were not significantly changed by the inhalation of nitrous oxide and sevoflurane. These observations suggest that the combination of sevoflurane and nitrous oxide induces an inflammatory response (increased production of IL-1β, IL-8 and MCP-1) and suppresses the anti-inflammatory response (reduced production of IL-12p70) in the local milieu of the airway. Thus, the combination of these compounds should be carefully administered for anesthesia.

Correspondence to: Professor Isao Nagaoka, Department of Host Defense and Biochemical Research, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
E-mail: nagaokai@med.juntendo.ac.jp

Key words: nitrous oxide, sevoflurane, airway epithelium, cytokine, chemokine

Introduction

Globally, >200 million major surgical procedures are undertaken annually (1), and nitrous oxide is widely used in anesthesia, often administered at an inspired concentration of ~60-70% (2-5). Nitrous oxide has achieved marked longevity as an anesthetic, having been used widely since 1844 (6). However, the low toxicity of modern anesthetic agents and the accumulating evidence on the adverse effects of nitrous oxide render the continued use of nitrous oxide in anesthesia controversial (7-10). A number of the adverse effects of nitrous oxide result from the irreversible inactivation of vitamin B_{12}, which inhibits methionine synthase, folate metabolism and deoxyribonucleic acid synthesis (7,8). This mechanism explains the megaloblastic anemia and neurologic toxicity associated with prolonged nitrous oxide administration (11,12), and the possible risk of teratogenicity, immunodeficiency and impaired wound healing (7,13,14). In addition, the inhibition of methionine synthase is associated with increased plasma homocysteine concentrations (15,16) which may increase the risk of post-operative cardiovascular complications (15). Furthermore, nitrous oxide impairs cerebral blood flow-activity coupling (10), and worsens the air space conditions (such as pneumothorax and air embolism) and bowel distension (8,9). Nitrous oxide is also a risk factor for post-operative nausea and vomiting (17,18), which are common, troublesome and costly complications of anesthesia (19).

As a weak anesthetic, nitrous oxide is often administered with other intravenous or volatile anesthetic agents (such as propofol and sevoflurane, respectively) as 60-70% of the inspired gas mixture containing 30-40% oxygen. Supplementation of oxygen during surgery potentially reduces the risk of wound infection (20,21), nausea and vomiting (22), which contribute to the duration of the hospital stay and cost of care.

Despite the adverse effects that may result directly from nitrous oxide, it is almost routinely used in patients undergoing

surgery (23). Notably, nitrous oxide has been reported to suppress the activation of neutrophils, an important type of inflammatory cell, *in vitro* (24,25). However, the effects of nitrous oxide on the inflammatory reaction in the respiratory tract remain to be elucidated. Thus, in the present study, the effects of nitrous oxide on the *in vivo* production of inflammatory cytokines and chemokines by airway epithelium were evaluated by combining nitrous oxide with other anesthetic agents (intravenous propofol and volatile sevoflurane).

Materials and methods

Subjects. In total, 52 females [age range, 22-72; mean age ± standard deviation (SD), 55.1±11.5 years] classified as American Society of Anesthesiologists Physical Status category I-II and undergoing simple or segmental mastectomy were recruited in this study. The study protocol was approved by the Local Ethics Committee of Juntendo University Nerima Hospital (Tokyo, Japan) and conducted from March 2008 to June 2009 in accordance with the principles of the amended Declaration of Helsinki and the Ethical Guidelines for Epidemiological Research (http://aje.oxfordjournals.org/content/170/11/1451.full). Subjects provided written informed consent prior to participating in the study. None of subjects received premedication.

The exclusion criteria included pregnancy, neurologic or psychiatric disease, cardiac disease classified as New York Heart Association classes II-IV (http://www.abouthf.org/questions_stages.htm), pre-operative severe impairment of respiratory function (such as a vital capacity or a forced expiratory volume in one second of <50% of that predicted), and pre-existing coagulopathy or thrombocytopenia. Subjects that exhibited systemic or local active infections (either clinically defined or evidenced by elevated C-reactive protein levels, leukocytosis or body temperature >38˚C) were also excluded.

Subjects were randomly assigned to a sevoflurane group (n=26) without (n=13) or with nitrous oxide (n=13), and a propofol group (n=26) without (n=13) or with nitrous oxide (n=13), using a list of random numbers generated by computer software (Microsoft Excel; Microsoft Corporation, Redmond, WA, USA).

Study protocols. Protocol 1: Induction of anesthesia was initiated with intravenous injection of propofol (1-2 mg/kg; AstraZeneca, Osaka, Japan), vecuronium bromide (0.1 mg/kg; MDS, Tokyo, Japan) and remifentanil (0.15-0.3 μg/kg/min; Janssen Pharmaceutical K.K., Tokyo, Japan). Subsequently, each subject was intubated with an endotracheal tube and their end-tidal CO_2 was verified. Anesthesia was maintained with the inhalation of sevoflurane (0.8-1.0 minimum alveolar concentration; Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) and intravenous infusion of remifentanil (0.15-0.3 μg/kg/min).

Protocol 2: Induction of anesthesia was initiated with intravenous infusion of propofol (using a target-controlled infusion technique with a target concentration of 3-4 μg/ml), vecuronium (0.1 mg/kg) and remifentanil (0.15-0.3 μg/kg/min). Subsequently, each subject was intubated with an endotracheal tube and their end-tidal CO_2 was verified. Anesthesia was maintained with intravenous infusion of propofol (target concentration 2-4 μg/ml) and remifentanil (0.15-0.3 μg/kg/min).

For nitrous oxide-based anesthesia, subjects were administered with a gas mixture of 60% nitrous oxide and 40% oxygen (O_2 2 l/min + nitrous oxide 3 l/min). For nitrous oxide-free anesthesia, subjects were administered with a gas mixture of 40% oxygen and 60% nitrogen (O_2 1 l/min + air containing 80% nitrogen and 20% oxygen 3 l/min).

An endotracheal tube (Rüsch® endotracheal tube; Teleflex Medical Sdn. Bhd., Kamunting, Malaysia) was inserted in all of the subjects. The position of the endotracheal tube was confirmed by a fiberoptic bronchoscopy (Portable Intubation Fiberscope FI-10RBS; Pentax, Tokyo, Japan). The subjects were ventilated by pressure-controlled ventilation with 4-cm H_2O positive end-expiratory pressure, and peak inspiratory pressure was maintained at >20 cm H_2O with a tidal volume of 7-10 ml/kg, and the fraction of inspired oxygen was maintained at 0.4. The oxygen saturation was adjusted to >97% and the respiratory rate was adjusted to maintain normocapnia (normal arterial carbon dioxide pressure).

For post-operative pain management, all the subjects were administered with 2 μg/kg of fentanyl (Daiichi Sankyo Company, Ltd., Tokyo, Japan) prior to the discontinuation of anesthetic agents.

Electrocardiogram, oxygen saturation, non-invasive blood pressure, end-tidal CO_2 pressure, esophageal body temperature and urine output were monitored during anesthesia and recorded every 5 min. Crystalloid solution was used for infusion, in accordance with the clinical needs of the patients. None of the subjects required blood transfusion.

Bronchoscopic microsampling. Epithelial lining fluid (ELF) was obtained using the bronchoscopic microsampling method prior to mastectomy (as the pre-operative level) and following mastectomy (as the post-operative level). A bronchofiberscope was inserted into the trachea through an endotracheal tube and placed at the bifurcation. A bronchoscopic microsampling probe (BC-402C; Olympus, Tokyo, Japan) was inserted into the right main bronchus through the channel of the bronchofiberscope; the probe consisted of a 2.6-mm outer-diameter polyethylene sheath and an inner 1.9-mm-diameter cotton probe (length, 20 mm) attached to a stainless steel guidewire. The inner probe was advanced gently 2-3 cm from the bifurcation into the right main bronchus until it made contact with the mucosal surface. ELF was obtained from the mucosal surface under direct observation. Subsequently, the inner probe was withdrawn, and the probe was sectioned at 30 mm from its tip and stored at -80˚C until analysis.

Measurement of cytokines. The probe was weighed and mixed with 500 μl saline by vortexing for 1 min, and then the solution was recovered. Subsequently, the probe was dried and reweighed to estimate the recovered ELF, which was calculated by subtracting the weight of the dried probes from that of the wet probes (the difference between the weight of the wet probe and the dried probe was ~10 mg), and the dilution factor was also calculated (26).

In the sevoflurane groups without or with nitrous oxide, inflammatory cytokine and chemokine levels in the ELF were measured using the cytometric bead array Human Inflammation kit and Human Chemokine kit (Becton-Dickinson, Franklin

Table I. Patient characteristics and surgical data.

Patient characteristics	Sevoflurane group (n=26)		Propofol group (n=26)	
	Air (n=13)	Nitrous oxide (n=13)	Air (n=13)	Nitrous oxide (n=13)
Age (years)	56.8±3.9	51.9±11.7	55.9±11.0	55.5±9.8
Actual weight (kg)	52.6±7.3	57.0±7.0	55.7±9.5	55.1±9.2
Height (cm)	155.3±4.4	155.4±4.5	154.3±6.3	158.7±3.3
Body mass index (kg/m^2)	21.8±2.6	21.5±4.8	23.3±3.0	21.9±3.7
Duration of surgery (min)	135.2±44.4	144.8±50.3	142.2±40.0	147.5±34.5
Duration of anesthesia (min)	183.8±43.3	195.2±53.4	194.1±44.8	193.9±36.8
Loss of blood (g)	99.5±109.9	83.5±66.7	50.8±28.3	65.9±30.7

Data are expressed as the mean ± SD.

Lakes, NJ, USA). The Human Inflammation kit included six fluorescently-distinguishable capture microbeads coated with antibodies against the following six analytes: Tumor necrosis factor (TNF)-α, and interleukin (IL)-1β, IL-6, IL-8, IL-10 and IL-12p70. The Human Chemokine kit included five fluorescently-distinguishable capture microbeads coated with antibodies against the following five analytes: IL-8, regulated upon activation normal T cell expressed and presumably secreted (RANTES), monokine induced by interferon γ (MIG), monocyte chemotactic protein-1 (MCP-1) and interferon γ-inducible protein 10 (IP-10). These methods detect cytokines and chemokines bound onto the microbeads by using an enzyme-linked immunosorbent assay (ELISA). The minimum quantifiable levels of cytokines and chemokines detected with the Human Inflammation and Chemokine kits were 20 and 10 pg/ml, respectively.

In the propofol groups with or without nitrous oxide, IL-6 and IL-8 levels in ELF were quantified by a sandwich ELISA using a Ready-Set-Go kit (eBioscience, San Diego, CA, USA) and a DuoSet ELISA Development kit (R&D Systems, Minneapolis, MN, USA), respectively. The detection limits of IL-6 and IL-8 detected with the ELISAs were 2 and 31.2 pg/ml, respectively.

Statistical analysis. Data are expressed as the mean ± SD and were statistically evaluated using the Wilcoxon signed-rank test and the Mann-Whitney U test. Differences were considered to be indicated as statistically significant when $P<0.05$. Analyses were performed with the statistical software program JMP® 7 (SAS Institute Inc., Cary, NC, USA).

Results

Subject characteristics. Table I shows the characteristics and surgical operation data of the 52 subjects divided into the four experimental groups. Subjects were evenly distributed among the four groups (sevoflurane groups with or without nitrous oxide, and propofol groups with or without nitrous oxide) based on their age, weight, height, body mass index, duration of surgery and anesthesia, and amount of blood lost. All the subjects were extubated following the surgery in the operating room and the post-operative course was uneventful.

Cytokine and chemokine levels in the ELF of the sevoflurane groups. As shown in Fig. 1, the levels of IL-1β, IL-8 and MCP-1 were significantly increased by the inhalation of nitrous oxide with sevoflurane (post-operative) compared with the baseline (pre-operative) ($P<0.05$). By contrast, the levels of IL-1β, IL-8 and MCP-1 were not significantly changed by the inhalation of air with sevoflurane (post-operative) compared with the baseline (pre-operative).

Of note, the levels of IL-12p70 were significantly reduced by the inhalation of sevoflurane with air (post-operative) compared with the baseline (pre-operative) ($P<0.01$). However, the levels of IL-12p70 were not significantly reduced by the inhalation of nitrous oxide with sevoflurane (post-operative) compared with the baseline (pre-operative).

The levels of IL-6, TNF-α, IL-10, RANTES, MIG and IP-10 were not significantly changed by the inhalation of nitrous oxide or air with sevoflurane (post-operative) compared with the baseline (pre-operative).

IL-8 and IL-6 levels in the ELF of the propofol groups. As shown in Fig. 2, the levels of IL-8 were not significantly changed by the inhalation of nitrous oxide with propofol anesthesia (post-operative) compared with the baseline levels (pre-operative). Similarly, the levels of IL-8 were not affected by the inhalation of air with propofol anesthesia (post-operative) compared with the baseline levels (pre-operative), as observed with sevoflurane anesthesia. In the propofol group with or without nitrous oxide, IL-6 was not detected in the ELF studied.

Discussion

A number of studies have shown that airway epithelial cells express and secrete various inflammatory and immune molecules, including cytokines (TNF-α, IL-1β and IL-6) and chemokines (IL-8 and MCP-1) (27-29). TNF-α, IL-1β and IL-6 function as proinflammatory molecules, whereas IL-8 and MCP-1 act as chemoattractants that are responsible for the recruitment of effector cells such as neutrophils and monocytes (30,31). Moreover, IL-12 is a strong inducer of interferon-γ and is important for the development of T-helper type I cells (32). Through the expression and production of

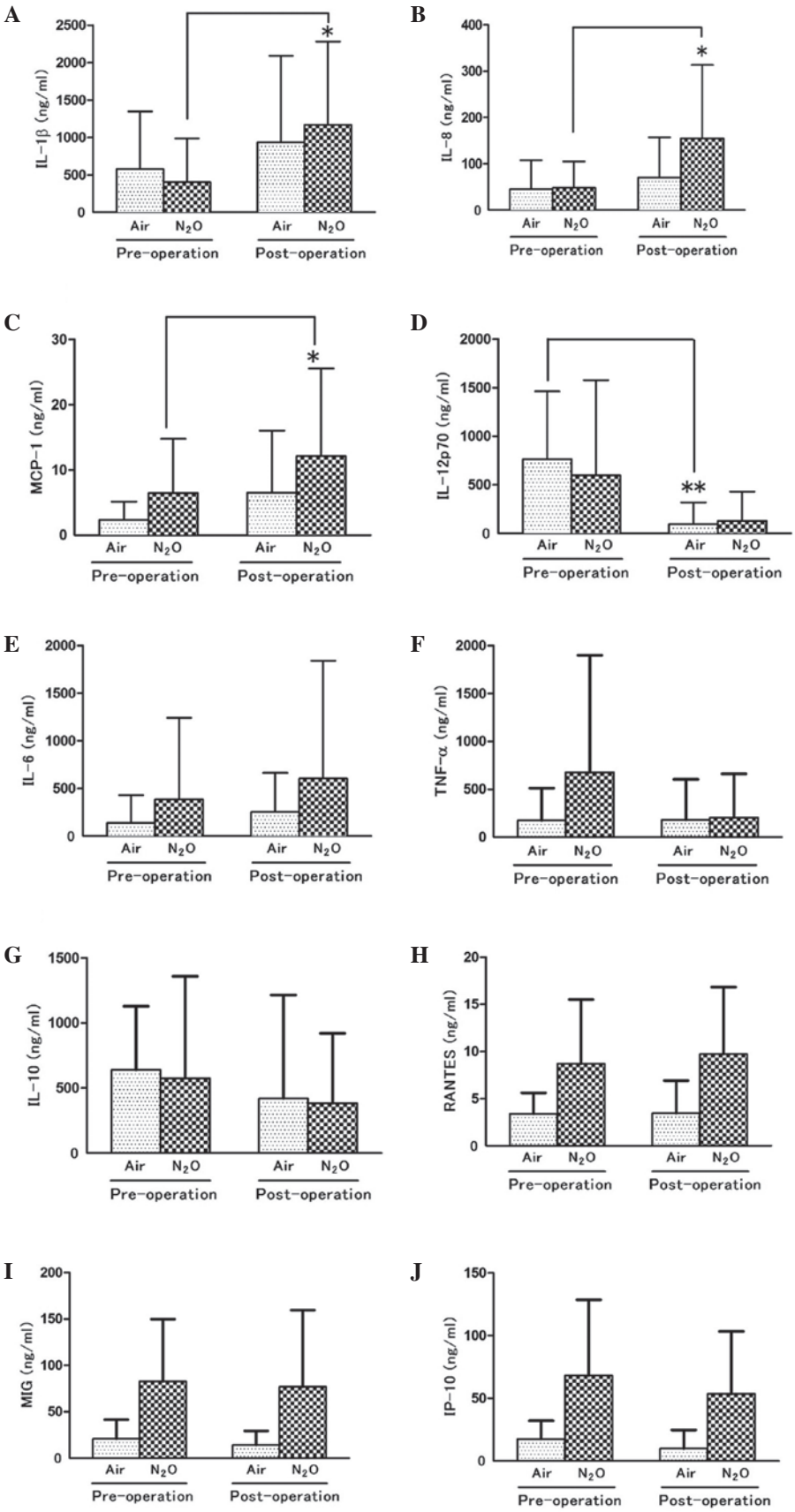

Figure 1. The levels of cytokines and chemokines in the ELF prior to and following the inhalation of sevoflurane with or without nitrous oxide. The levels of (A) IL-1β, (B) IL-8, (C) MCP-1, (D) IL-12p70, (E) IL-6, (F) TNF-α, (G) IL-10, (H) RANTES, (I) MIG and (J) IP-10 were measured with a CBA system using ELF recovered from patients undergoing mastectomy prior to (pre-operative) and following (post-operative) the inhalation of sevoflurane with (N_2O) or without nitrous oxide (air). Data are expressed as the mean ± SD, and compared between the pre- and post-operative levels. *P<0.05, **P<0.01. IL, interleukin; MCP-1, monocyte chemotactic protein-1; TNF, tumor necrosis factor; RANTES, regulated upon activation normal T cell expressed and presumably secreted; MIG, monokine induced by interferon γ; IP-10, interferon γ-inducible protein 10; ELF, epithelial lining fluid; CBA, cytometric bead array.

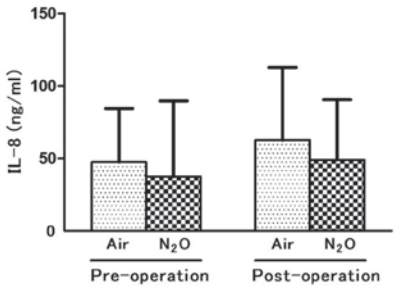

Figure 2. The levels of IL-8 in the ELF prior to and following the propofol-based anesthesia with or without nitrous oxide. The levels of IL-8 were measured with a sandwich ELISA using ELF recovered from patients undergoing a mastectomy prior to (pre-operative) and following (post-operative) the propofol-based anesthesia with (N_2O) or without nitrous oxide (air). Data are expressed as the mean ± SD. ELISA, enzyme-linked immunosorbent assay; ELF, epithelial lining fluid.

these molecules, the airway epithelium is important in the initiation and exacerbation of inflammatory responses within the airways.

Volatile anesthetics, such as sevoflurane and isoflurane, have been shown to attenuate the cardiac injury caused upon ischemia-reperfusion (33) and to exert anti-inflammatory action in a model of endotoxin-induced lung injury *in vivo* (34). Furthermore, *in vitro* studies have demonstrated that sevoflurane exhibited direct anti-inflammatory and antinecrotic effects on cultured human kidney cells following ischemia-reperfusion injury (35). In addition, sevoflurane has not only been shown to suppress the expression of inflammatory mediators such as MCP-1, macrophage inflammatory protein (MIP)-1β, MIP-2 and cytokine-induced neutrophil chemoattractant-1 and the adhesion molecule ICAM-1, but it has also been shown to reduce neutrophil adhesion to lipopolysaccharide-injured alveolar epithelial cells *in vitro* (36). In this context, one-lung ventilation (OLV) has been demonstrated to induce an increase in the inflammatory molecules TNF-α, IL-1β, IL-6, IL-8 and MCP-1 in propofol- and sevoflurane-anesthesized patients; however, the levels of inflammatory mediators following OLV, with the exception of IL-1β, were significantly lower in the sevoflurane group compared with those in the propofol group (29). Thus, sevoflurane likely exerts anti-inflammatory activities on various types of cells. Notably, it has been suggested that sevoflurane exerts an anti-inflammatory action via the mechanisms involving the reduction of mRNA and protein levels of the proinflammatory transcription factors NF-κB and AP-1 (35,37).

Nitrous oxide is widely used as an anesthetic in humans. Its short-acting analgesic properties reduce the requirement for the more potent anesthetics combined. Thus, nitrous oxide is generally considered to be a relatively safe anesthetic. Nevertheless, a number of adverse effects are known, including megaloblastic anemia, homocysteinemia and possible risks for atherosclerosis, thrombosis, cognitive dysfunction, neurotoxicity, teratogenicity, increased intracranial pressure, hypoxia, post-operative nausea and vomiting, and immunosuppression.

Although controversial, *in vitro* and *in vivo* studies have demonstrated that nitrous oxide reduces neutrophil motility and chemotaxis as well as the oxidative response (25,38-40,41-43). Furthermore, nitrous oxide depresses the expression of endogenous sugar receptors on polymorphonuclear leukocytes and impairs intracellular signaling in leukocytes, resulting in the interference of the leukocyte adhesion-activation cascade (44,45). These observations clearly indicate that nitrous oxide acts as an anti-inflammatory molecule.

Notably, in the present study, it was identified that the levels of IL-1β, IL-8 and MCP-1 in ELF were significantly increased by the inhalation of sevoflurane with nitrous oxide, although the levels of IL-1β, IL-8 and MCP-1 were not significantly changed by the inhalation of sevoflurane with air. Furthermore, the levels of IL-12p70 were not significantly reduced by the inhalation of nitrous oxide with sevoflurane, although the levels of IL-12p70 were significantly reduced by the inhalation of sevoflurane with air. These observations suggest that the aforementioned anti-inflammatory actions of sevoflurane and nitrous oxide are abrogated by the combination of these compounds, and the inhalation of nitrous oxide likely induces the inflammatory response (increased production of IL-1β, IL-8 and MCP-1) or suppresses the anti-inflammatory response (reduced production of IL-12p70) under the sevoflurane anesthesia. Of note, this possibility is supported by the finding that the IL-8 levels were not increased by the inhalation of nitrous oxide with propofol. Moreover, our hypothesis is reinforced by the following findings from a previous study: The number of leukocytes rolling along and firmly adherent to the endothelial wall of cerebral venules were significantly increased in animals anesthetized with nitrous oxide in combination with isoflurane or halothane (a halogenated fluorine-containing anesthetic similar to sevoflurane), compared with isoflurane or halothane alone; and the combination of isoflurane or halothane and nitrous oxide significantly increased the number of neutrophils invading the brain parenchyma in animals compared with that of isoflurane or halothane alone (46).

In the present study, it was demonstrated that several cytokine and chemokine levels were increased in ELF by the inhalation of nitrous oxide with sevoflurane (following the surgery of mastectomy). Notably, it has already been reported that the levels of cytokines and chemokines (TNF-α, IL-8, IL-1β, IL-10 and IL-12p70) are increased in the ELF, however, these cytokine/chemokine levels remained undetectable in the plasma even following OLV and thoracic surgery (lobectomy and partial lung resection) (47). Thus, we hypothesize that the inflammatory response observed in the present study is restricted to the local airway surface. In conclusion, findings of the present study suggest that nitrous oxide and sevoflurane should be carefully administered for anesthesia, as the combination of these compounds induces an inflammatory response in the local milieu of the airway (as evidenced by enhanced cytokine/chemokine expression).

References

1. Weiser TG, Regenbogen SE, Thompson KD, *et al*: An estimation of the global volume of surgery: a modelling strategy based on available data. Lancet 372: 139-144, 2008.
2. Fisher DM and Zwass MS: MAC of desflurane in 60% nitrous oxide in infants and children. Anesthesiology 76: 354-356, 1992.
3. Gupta K, Vohra V and Sood J: The role of magnesium as an adjuvant during general anaesthesia. Anaesthesia 61: 1058-1063, 2006.
4. Myles PS, Leslie K, Chan MT, *et al*: ENIGMA Trial Group. Avoidance of nitrous oxide for patients undergoing major surgery: a randomized controlled trial. Anesthesiology 107: 221-231, 2007.

5. Myles PS, Leslie K, Peyton P, *et al*; ANZCA Trials Group: Nitrous oxide and perioperative cardiac morbidity (ENIGMA-II) Trial: rationale and design. Am Heart J 157: 488-494, 2009.
6. Urman RD and Desai SP: History of anesthesia for ambulatory surgery. Curr Opin Anaesthesiol 25: 641-647, 2012.
7. Nunn JF: Clinical aspects of the interaction between nitrous oxide and vitamin B12. Br J Anaesth 59: 3-13, 1987.
8. Myles PS, Leslie K, Silbert B, Paech M and Peyton P: A review of the risks and benefits of nitrous oxide in current anaesthetic practice. Anaesth Intensive Care 32: 165-172, 2004.
9. Akca O, Lenhardt R, Fleischmann E, *et al*: Nitrous oxide increases the incidence of bowel distension in patients undergoing elective colon resection. Acta Anaesthesiol Scand 48: 894-898, 2004.
10. Kaisti KK, Langsjo JW, Aalto S, *et al*: Effects of sevoflurane, propofol, and adjunct nitrous oxide on regional cerebral blood flow, oxygen consumption, and blood volume in humans. Anesthesiology 99: 603-613, 2003.
11. Marie RM, Le Biez E, Busson P, *et al*: Nitrous oxide anesthesia-associated myelopathy. Arch Neurol 57: 380-382, 2000.
12. Schilling RF: Is nitrous oxide a dangerous anesthetic for vitamin B12-deficient subjects. JAMA 255: 1605-1606, 1986.
13. Parbrook GD: Leucopenic effects of prolonged nitrous oxide treatment. Br J Anaesth 39: 119-127, 1967.
14. Mazze RI, Wilson AI, Rice SA and Baden JM: Reproduction and fetal development in rats exposed to nitrous oxide. Teratology 30: 259-265, 1984.
15. Badner NH, Beattie WS, Freeman D and Spence JD: Nitrous oxide-induced increased homocysteine concentrations are associated with increased postoperative myocardial ischemia in patients undergoing carotid endarterectomy. Anesth Analg 91: 1073-1079, 2000.
16. Badner NH, Drader K, Freeman D and Spence JD: The use of intraoperative nitrous oxide leads to postoperative increases in plasma homocysteine. Anesth Analg 87: 711-713, 1998.
17. Tramer M, Moore A and McQuay H: Meta-analytic comparison of prophylactic antiemetic efficacy for postoperative nausea and vomiting: Propofol anaesthesia versus omitting nitrous oxide versus total i.v. anaesthesia with propofol. Br J Anaesth 78: 256-259, 1997.
18. Apfel CC, Korttila K, Abdalla M, *et al*: A factorial trial of six interventions for the prevention of postoperative nausea and vomiting. N Engl J Med 350: 2441-2451, 2004.
19. Macario A, Weinger M, Carney S and Kim A: Which clinical anesthesia outcomes are important to avoid? The perspective of patients. Anesth Analg 89: 652-658, 1999.
20. Greif R, Akca O, Horn EP, Kurz A and Sessler DI: Supplemental perioperative oxygen to reduce the incidence of surgical wound infection. N Engl J Med 342: 161-167, 2000.
21. Belda FJ, Aguilera L, Garcia de la Asunción J, *et al*: Supplemental perioperative oxygen and the risk of surgical wound infection. JAMA 294: 2035-2042, 2005.
22. Grief R, Laciny S, Rapf B, Hickle RS and Sessler DI: Supplemental oxygen reduces the incidence of postoperative nausea and vomiting. Anesthesiology 91: 1246-1252, 1999.
23. Henderson KA, Raj N and Hall JE: The use of nitrous oxide in anaesthetic practice: a questionnaire survey. Anaesthesia 57: 1155-1158, 2002.
24. Schneemilch CE, Hachenberg T, Ansorge S, Ittenson A and Bank U: Effects of different anaesthetic agents on immune cell function in vitro. Eur J Anaesthesiol 22: 616-623, 2005.
25. Moudgil GC, Gordon J and Forrest JB: Comparative effects of volatile anaesthetic agents and nitrous oxide on human leucocyte chemotaxis in vitro. Can Anaesth Soc J 31: 631-637, 1984.
26. Ishizaka A, Watanabe M, Yamashita T, *et al*: New bronchoscopic microsample probe to measure the biochemical constituents in epithelial lining fluid of patients with acute respiratory distress syndrome. Crit Care Med 29: 896-898, 2001.
27. Simon RH and Paine R III: Participation of pulmonary alveolar epithelial cells in lung inflammation. J Lab Clin Med 126: 108-118, 1995.
28. Takizawa H: Airway epithelial cells as regulators of airway inflammation. Int J Mol Med 1: 367-378, 1998.
29. De Conno E, Steurer MP, Wittlinger M, *et al*: Anesthetic-induced improvement of the inflammatory response to one-lung ventilation. Anesthesiology 110: 1316-1326, 2009.
30. Vozzelli MA, Mason SN, Whorton MH and Auten RL Jr: Antimacrophage chemokine treatment prevents neutrophil and macrophage influx in hyperoxia-exposed newborn rat lung. Am J Physiol Lung Cell Mol Physiol 286: L488-L493, 2004.
31. Beck-Schimmer B, Schwendener R, Pasch T, Reyes L, Booy C and Schimmer RC: Alveolar macrophages regulate neutrophil recruitment in endotoxin-induced lung injury. Respir Res 6: 61-66, 2005.
32. Brunda MJ: Interleukin-12. J Leukoc Biol 55: 280-288, 1994.
33. Tanaka K, Ludwig LM, Kersten JR, Pagel PS and Warltier DC: Mechanisms of cardioprotection by volatile anesthetics. Anesthesiology 100: 707-721, 2004.
34. Reutershan J, Chang D, Hayes JK and Ley K: Protective effects of isoflurane pretreatment in endotoxin-induced lung injury. Anesthesiology 104: 511-517, 2006.
35. Lee HT, Kim M, Jan M and Emala CW: Anti-inflammatory and antinecrotic effects of the volatile anesthetic sevoflurane in kidney proximal tubule cells. Am J Physiol Renal Physiol 291: 67-78, 2006.
36. Suter D, Spahn DR, Blumenthal S, Reyes L, Booy C, Z'Graggen BR and Beck-Schimmer B: The immunomodulatory effect of sevoflurane in endotoxin-injured alveolar epithelial cells. Anesth Analg 104: 638-645, 2007.
37. Loop T, Scheiermann P, Doviakue D, *et al*: Sevoflurane inhibits phorbol-myristate-acetate-induced activator protein-1 activation in human T lymphocytes in vitro: potential role of the p38-stress kinase pathway. Anesthesiology 101: 710-721, 2004.
38. Frohlich D, Rothe G, Wittmann S, Schmitz G, Schmid P, Taeger K and Hobbhahn J: Nitrous oxide impairs the neutrophil oxidative response. Anesthesiology 88: 1281-1290, 1998.
39. Hill GE, English JB, Stanley TH, Kawamura R, Loeser EA and Hill HR: Nitrous oxide and neutrophil chemotaxis in man. Br J Anaesth 50: 555-558, 1978.
40. Kripke BJ, Kupferman A and Luu KC: Suppression of chemotaxis to corneal inflammation by nitrous oxide. Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zhi 20: 302-310, 1987.
41. Nunn JF and O'Morain C: Nitrous oxide decreases motility of human neutrophils in vitro. Anesthesiology 56: 45-48, 1982.
42. Welch WD: Effect of enflurane, isoflurane, and nitrous oxide on the microbicidal activity of human polymorphonuclear leukocytes. Anesthesiology 61: 188-192, 1984.
43. Welch WD and Zaccari J: Effect of halothane and N_2O on the oxidative activity of human neutrophils. Anesthesiology 57: 172-176, 1982.
44. Bardosi L, Bardosi A and Gabius HJ: Changes of expression of endogenous sugar receptors by polymorphonuclear leukocytes after prolonged anaesthesia and surgery. Can J Anaesth 39: 143-150, 1992.
45. Frohlich D, Rothe G, Schmitz G and Taeger K: Nitrous oxide impairs the signaling of neutrophils downstream of receptors. Toxicol Lett 100-101: 121-127, 1998.
46. Lehmberg J, Waldner M, Baethmann A and Uhl E: Inflammatory response to nitrous oxide in the central nervous system. Brain Res 30: 1246: 88-95, 2008.
47. Sugasawa Y, Yamaguchi K, Kumakura S, Murakami T, Suzuki K, Nagaoka I and Inada E: Effects of sevoflurane and propofol on pulmonary inflammatory responses during lung resection. J Anesth 26: 62-69, 2012.