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

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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 sevoflurane and air. 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.

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 B12, 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 CO₂ 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 CO₂ 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 (O₂ 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 (O₂ 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 H₂O positive end-expiratory pressure, and peak inspiratory pressure was maintained at >20 cm H₂O 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 (Daichi Sankyo Company, Ltd., Tokyo, Japan) prior to the discontinuation of anesthetic agents.

Electrocardiogram, oxygen saturation, non-invasive blood pressure, end-tidal CO₂ 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...
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

Table I. Patient characteristics and surgical data.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Sevoflurane group (n=26)</th>
<th>Propofol group (n=26)</th>
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<tbody>
<tr>
<td></td>
<td>Air (n=13)</td>
<td>Nitrous oxide (n=13)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.8±3.9</td>
<td>51.9±11.7</td>
</tr>
<tr>
<td>Actual weight (kg)</td>
<td>52.6±7.3</td>
<td>57.0±7.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155.3±4.4</td>
<td>155.4±4.5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.8±2.6</td>
<td>21.5±4.8</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
<td>135.2±44.4</td>
<td>144.8±50.3</td>
</tr>
<tr>
<td>Duration of anesthesia (min)</td>
<td>183.8±43.3</td>
<td>195.2±53.4</td>
</tr>
<tr>
<td>Loss of blood (g)</td>
<td>99.5±109.9</td>
<td>83.5±66.7</td>
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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), 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).
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 (N₂O) 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.
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 antinociceptive 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)-β, IL-8 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-anesthetized 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


