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Short communication

DNA damage in patients who underwent minimally invasive surgery under inhalation or intravenous anesthesia

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ABSTRACT

Recent studies have demonstrated the genotoxicity of anesthetics in patients who have undergone surgery and in personnel who are occupationally exposed to anesthetics. However, these findings are controversial. Herein, we used the comet assay (single-cell gel electrophoresis) to investigate the genotoxic effects of two volatile compounds [isoflurane (ISF) and sevoflurane (SVF)] that are used in inhalation anesthesia, and of one intravenous (iv) anesthetic compound [propofol (PF)]. The groups consisted of 45 patients who underwent minimally invasive surgery that lasted at least 2 h. Patients were classified as physical status I using the criteria of the American Society of Anesthesiologists (ASA) and were randomly allocated to receive ISF, SVF or PF anesthesia. Venous blood samples were collected at three time points as follows: before the premedication and the induction of anesthesia (T_0); 2 h after the beginning of anesthesia (T_1); and on the day following surgery (T_2). DNA damage (strand breaks and alkali-labile sites) was evaluated in peripheral blood lymphocytes. For each patient, one hundred nucleoids were analyzed per time point using a semi-automated image system. Patients did not differ with respect to their demographic characteristics, the duration of surgery, or the total doses of intraoperative drugs. The amount of DNA damage was not different among the three groups before anesthesia (T_0). No statistically significant ($p > 0.05$) increase in DNA damage was detected during (T_1) or after anesthesia (T_2) using three different protocols (ISF, SVF or PF). In conclusion, general anesthesia with inhaled ISF and SVF or iv PF did not induce DNA strand breaks or alkali-labile sites in peripheral lymphocytes. Therefore, our results show that the genotoxic risk of these anesthetics, for healthy patients undergoing minimally invasive otorhinological surgery, is low or even absent.

1. Introduction

Every year, approximately 100 million people undergo surgery worldwide. Although the safety of anesthesia has dramatically improved, there are still some adverse effects and unexpected outcomes. Among the inhaled anesthetic gases, the halogenated gases isoflurane (ISF) and sevoflurane (SVF) are the most widely used in general anesthesia. ISF was first synthesized in 1965 and was approved for clinical use several years later. Corbett [1] has suggested that this anesthetic may produce liver tumors in rats. However, these findings were not confirmed in another study [2]. The advantages of ISF are its low rate of metabolism and low solubility, which decrease the induction and recovery times. However, there are controversial results regarding its genotoxic and mutagenic effects *in vitro* and *in vivo* [3–7].

SVF was developed in the late 1960s, but was only approved for clinical use in 1995. SVF can degrade to form a toxic product known as Compound A, which increases the frequency of sister chromatid exchange (SCE) in Chinese hamster ovary cells (CHO) [8]. On the other hand, Compound A was not found to be mutagenic in the Ames test [9]. SVF has a low blood-gas partition coefficient, which allows rapid induction and awakening. Because of its sweet smell, SVF is used for anesthesia induction in both adults and children. Conflicting data have been presented concerning the genotoxic and mutagenic potential of SVF *in vitro*, in animals and in patients [10–14].

Propofol (PF), an intravenous sedative-hypnotic agent, was commercially introduced in the United States in 1989. PF is the first of a new class of intravenous anesthetics: the alkylphenols. PF is characterized by a phenolic structure that is similar to that of

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α-tocopherol and has antioxidant properties [15]. Few studies have evaluated the genotoxic potential of PF. However, negative results have been observed when PF was administered to children [16] and adults undergoing surgery [17,18].

The genotoxicity of anesthetic drugs has been investigated especially in cells from patients with mild systemic disease, and/or patients who underwent invasive or major surgeries. To our knowledge, no previous studies have compared the ISF-, SVF- or PF-induced DNA damage in healthy patients undergoing minimally invasive surgeries, such as tympanoplasty and septoplasty. Therefore, the present study was designed to evaluate and compare the genotoxic potentials of these three anesthetics (two volatile and one intravenous) in adult patients who underwent elective minimally invasive otorhinological surgery.

2. Patients and methods

2.1. Study population

The Ethical Committee for Human Research of the Botucatu Medical School (UNESP) approved the protocol (423/2006) that was used in the present study. After signing the informed consent form, all of the patients completed a detailed questionnaire about their lifestyle, health status and previous exposure to environmental pollutants. In this study, we enrolled 45 subjects of both sexes, aged from 18 to 40 years old who were scheduled for elective surgery lasting at least 2 h and who were classified by the American Society of Anesthesiologists (ASA) as physical status I patients (healthy patient with no disease other than a surgical abnormality). Only patients undergoing minimally invasive surgical procedures, such as septoplasty and tympanoplasty were included to minimize the possible influence of trauma on the comet assay results. Smokers, alcoholics, obese subjects, and those who had recently received radiation, medicines and/or antioxidant supplements were excluded from the study. Patients were randomly allocated into the following three groups: ISF ($n = 15$), SVF ($n = 15$) and PF ($n = 15$).

2.2. Anesthesia procedure

The following standard clinical monitoring was performed: electrocardiogram, peripheral oxygen saturation (SpO_2), non-invasive arterial pressure (systolic and diastolic), end-tidal CO_2 ($PETCO_2$) and end-tidal ISF and SVF. All patients were premedicated with intravenous (iv) benzodiazepine midazolam (3 mg). In the ISF and SVF groups, anesthesia was induced using the opioid fentanyl (5 μg/kg, iv) and the hypnotic propofol (2 mg/kg, iv) and maintained with ISF (Abbott) or SVF (Abbott) inhalation at approximately 1.0 minimum alveolar concentration equivalent to 1.2% and 1.9%, respectively. In the PF group (iv anesthesia), the induction of anesthesia was performed using fentanyl (5 μg/kg, iv) and propofol (Diprivan®). PF was administered by a computer-controlled infusion pump. The estimated plasma concentration of propofol was maintained at 3–5 μg/ml until the end of surgery. All groups received the neuromuscular blocker rocuronium bromide (0.6 mg/kg, iv). The lungs were mechanically ventilated using the volume-controlled mode with a tidal volume of 8 ml/kg of 40% oxygen (0.8 l/min) in air (1.2 l/min) and a respiratory rate of 10–12 breaths/min to maintain the $PETCO_2$ concentration at 30–35 mmHg. The effectiveness of anesthesia during maintenance was monitored by assessing the hemodynamic responses. Additional doses of fentanyl (2 μg/kg) and rocuronium (0.2 mg/kg) were administered if the patient was considered to be inadequately anesthetized.

2.3. Blood sampling

Venous blood samples were drawn from all patients at three time points as follows: before premedication and the induction of anesthesia (T_0), 2 h after the beginning of anesthesia (T_1) and on the day following surgery (T_2). Blood was collected in anticoagulant tubes, and all samples were coded and blindly analyzed.

2.4. Alkaline comet assay

The protocol used for lymphocyte isolation followed the general procedures that have already been published [19]. The comet assay was conducted according to the methodology that has been described by Singh et al. [20] and Tice et al. [21] with slight modifications [19]. Every step was performed under indirect light. Briefly, fresh lymphocytes (10 μl) were added to 120 μl of 0.5% low-melting-point agarose at 37 °C. The mixtures were layered on 1.5% normal-agarose precoated slides, covered with a coverslip and left at 4 °C for 5 min to allow the agarose to solidify. The coverslips were removed, and the slides were immersed in cold lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris at pH 10, 1% Triton X-100 and 10% DMSO) for at least 2 h. The slides were washed in phosphate-buffered saline (PBS) for 5 min and immersed in freshly prepared alkaline buffer (1 mM EDTA and 300 mM NaOH at pH > 13) in a horizontal electrophoresis tank. After a 40-min DNA unwinding period, electrophoresis was conducted at 25 V and 300 mA for 30 min. Following 15 min of neutralization with 0.4 M Tris (pH 7.5), the slides were fixed with absolute ethanol and stored at 4 °C. Prior to analysis, the slides were stained with 50 μl of ethidium bromide (20 μg/ml) and immediately analyzed using a fluorescent microscope at a magnification of 400×. Images from 100 nucleoids (two replicate samples) per time point per patient were scored using a semi-automated image analysis system (Comet Assay II, Perceptive Instruments, Haverhill, UK). The tail intensity (% DNA in the tail) was used to estimate the extent of DNA damage.

Table 1
Demographic data of the study groups (X ± SD or absolute number).

Characteristics	Groups			*p* Value
	Isoflurane	Sevoflurane	Propofol	
Age (years)	25.2 ± 7.1	28.0 ± 9.1	27.6 ± 9.9	0.64
Sex (male/female)	8/7	8/7	8/7	1.0
Weight (kg)	67.7 ± 12.8	66.4 ± 11.1	61.5 ± 9.7	0.29
Height (cm)	168.0 ± 8.7	167.3 ± 9.7	165.5 ± 6.9	0.72
Body mass index (kg/m^2)	23.9 ± 3.6	23.8 ± 4.1	22.5 ± 2.9	0.46

Table 2
Duration of surgery and total doses of the opioid and neuromuscular blocker in the groups (X ± SD or absolute number).

	Groups			*p* Value
	Isoflurane	Sevoflurane	Propofol	
Duration of surgery (min)	143.5 ± 36.9	163.3 ± 55.1	166.0 ± 39.7	0.33
Intraoperative anesthesia				
Fentanyl (μg)	442.7 ± 147.5	456.7 ± 89.9	516.7 ± 126.7	0.23
Rocuronium (mg)	45.1 ± 8.0	43.7 ± 7.7	47.2 ± 9.7	0.52

2.5. Statistical analysis

Demographic characteristics of the study population, the duration of surgery and intraoperative data were expressed as the means ± standard deviation (X ± SD) and were analyzed using the one-way ANOVA test. The Chi-square test was used to analyze differences between sexes. DNA damage was evaluated for each anesthetic among the three time points, and also among the three groups of anesthetics in each sampling time. Therefore, DNA damage data were grouped by anesthetic and blood sampling time. Since data did not show a normal distribution, non-parametric tests were used for statistical analyses: Friedman test was used to compare DNA damage within the same group of anesthetics (to compare the time points) whereas Kruskal-Wallis test was applied for comparing the three groups of anesthetics in each sampling time. DNA damage was expressed as the median with the 1st and 3rd quartiles. Significance was set at $p < 0.05$.

3. Results

No statistically significant differences ($p > 0.05$) were detected among the groups regarding the demographic characteristics of the patients, the duration of surgery and the total doses of intraoperative drugs (Tables 1 and 2). All hemodynamic and ventilatory parameters that were monitored during the surgical procedure were within the expected ranges (data not shown).

Table 3 presents the amount of DNA damage as depicted by the comet assay in each group of patients and at different blood sampling time points. No significant differences ($p > 0.05$) were observed among the three groups before, during or after anesthesia.

Table 3
DNA damage in peripheral blood lymphocytes collected at three time points (T_0, T_1 and T_2) from patients undergoing surgery with inhalation or intravenous anesthesia.

Group	DNA damage (% DNA in the comet tail)		
	T_0	T_1	T_2
Isoflurane	1.9 (0.9; 4.2)	2.2 (1.4; 4.6)	1.6 (0.8; 3.3)
Sevoflurane	1.1 (0.5; 1.7)	1.6 (0.6; 3.4)	1.3 (0.5; 2.3)
Propofol	1.4 (0.6; 3.8)	1.0 (0.4; 3.1)	0.8 (0.3; 6.4)

T_0: before induction of anesthesia, T_1: 2 h after the beginning of anesthesia, T_2: on the day following surgery. Medians (1st quartile; 3rd quartile). $p > 0.05$.

4. Discussion

Conflicting data about the genotoxic effects of anesthetics in patients undergoing surgery have been published and are probably the result of different experimental designs, the type and duration of surgery, and the characteristics of the patients (age, physical status, associated co-morbidities or diseases, and other drugs that were used during the surgical procedure). The present study showed that ISF, SVF and PF did not induce strand breaks or alkali-labile sites in lymphocytes from ASA I (healthy) patients undergoing minimally invasive surgeries, such as tympanoplasty and septoplasty.

Our results are in accordance with those of the International Agency for Research on Cancer, which classifies volatile anesthetics as Group 3, i.e., not classifiable with respect to their carcinogenicity to humans [22]. Previous studies have demonstrated that ISF is not genotoxic in the Ames test and in *Drosophila melanogaster* assays [3,23]. Similarly, ISF did not increase the frequency of sister chromatid exchange (SCE) in some cell lines [24,25]. Patients who underwent minor orthopedic surgery under ISF anesthesia did not exhibit an increased frequency of SCE [4]. Furthermore, in a recent study, we did not detect increases in oxidative/alkylated DNA damage as recognized by the FPG and endonuclease III enzymes in patients undergoing elective surgery with ISF anesthesia [7]. However, positive ISF genotoxicity has been observed in 12 patients classified as ASA I and II (patients with mild systemic disease) aged from 20 to 66 years, who underwent abdominal surgeries [5,26]. Similarly, a group of neurosurgical patients exhibited high levels of DNA damage 180 min after ISF-nitrous oxide (N_2O)-O_2 anesthesia, which decreased by the first postoperative day [27].

Previous studies have not shown SVF-mediated increases in DNA damage in ASA I and II patients undergoing invasive orthopedic surgery 15 min after the induction of anesthesia with 2% SVF in O_2. Nevertheless, DNA strand breaks have been detected on the first postoperative day [10]. In the same study, the authors observed increased oxidative DNA damage 15 min after the induction of anesthesia but not on the first day following surgery. SVF and ISF exhibited similar genotoxic responses in patients who underwent abdominal surgeries. In these patients, DNA damage increased at 60 and 120 min of anesthesia until the first day after anesthesia and returned to baseline values 3 days after surgery [26]. Reversible genotoxic effects of 1–1.5% SVF have been reported in breast cancer patients (stages II and III) who underwent mastectomy [13]. Contrarily, negative findings in the comet assay have been observed with lymphocytes treated with 10 mM of SVF *in vitro*, and in anesthetists (occupational exposure) [12]. Children (1–14 years old) who underwent minor surgical procedures under SVF (2.5–3%) in O_2/N_2O anesthesia do not display increased frequencies of SCE in lymphocytes that were collected at the end of surgery [11].

To minimize possible confounding factors, only non-smoking young adult patients with normal body mass indexes and no other associated-diseases were included in the present study. Patients were fasted for 8 h before underwent surgery. This is a short-term fasting, which is necessary to avoid regurgitation during anesthesia induction. There are a few studies regarding fasting and oxidative damage, but Lee et al. [28] have observed that prolonged fasting reduces urinary levels of lipid peroxidation products, without affecting biomarkers of oxidative DNA damage in healthy women. Similarly, Ramadan fasting did not affect oxidative stress parameters in healthy subjects, with the exception of a slightly reduction of lipid peroxidation in erythrocytes [29]. In our study, all patients underwent similar minimally invasive surgeries that lasted similar amounts of time and were administered the same drugs during anesthesia. The association between inflammation and oxidative stress is well documented [30]. Minimally invasive surgeries cause less tissue injury than major and invasive procedures, with lower release of inflammatory biomarkers [31,32].

The genotoxicity induced by polyfluorinated anesthetics, such as ISF, is believed to be associated with a direct interaction with DNA (possibly alkylation at the N-7 position of purines) or residual metabolism, which induces the formation of reactive products [6,10]. However, increases in oxidative and alkylated DNA damage, which are recognized by the FPG, have not been observed in patients who underwent surgery with ISF [7].

No mutagenicity of PF and its metabolites has been detected in *Salmonella typhimurium*, *Saccharomyces cerevisiae* [33] and CHO cells [34]. Similarly, no increase of SCE has been observed in children undergoing inguinal herniotomies and strabismus surgeries [16] nor has there been an increased level of chromosome aberrations (CA) observed in adult patients undergoing cardiac surgeries [17]. In a previous study, we showed that peripheral blood cells that are collected from patients during and after surgical procedures are not different with respect to the amount of DNA damage when evaluated using the comet assay [18]. These data support our present findings that PF anesthesia was not genotoxic. Its chemical structure, which is similar to that of phenol-based free radical scavengers such as vitamin E, may favor an antioxidant activity to prevent DNA damage.

In conclusion, general anesthesia with inhaled isoflurane and sevoflurane or with intravenous propofol does not induce DNA strand breaks or alkali-labile sites in human peripheral lymphocytes *in vivo*. Therefore, our results show that the genotoxic risk of these anesthetics, for healthy patients undergoing minimally invasive otorhinological surgery, is low or even absent.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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