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Isoflurane and Propofol Contribute to Increasing the Antioxidant Status of Patients During Minor Elective Surgery

A Randomized Clinical Study

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Abstract: Isoflurane is a volatile halogenated anesthetic used especially for anesthesia maintenance whereas propofol is a venous anesthetic utilized for anesthesia induction and maintenance, and reportedly an antioxidant. However, there are still controversies related to isoflurane-induced oxidative stress and it remains unanswered whether the antioxidant effects occur in patients under propofol anesthesia.

Taking into account the importance of better understanding the role of anesthetics on oxidative stress in anesthetized patients, the present study was designed to evaluate general anesthesia maintained with isoflurane or propofol on antioxidant status in patients who underwent minimally invasive surgeries.

We conducted a prospective randomized trial in 30 adult patients without comorbidities who underwent elective minor surgery (septoplasty) lasting at least 2 h admitted to a Brazilian tertiary hospital.

The patients were randomly allocated into 2 groups, according to anesthesia maintenance (isoflurane, $n = 15$ or propofol, $n = 15$). Peripheral blood samples were drawn before anesthesia (baseline) and 2-h after anesthesia induction.

The primary outcomes were to investigate the effect of either isoflurane or propofol anesthesia on aqueous plasma oxidizability and total antioxidant performance (TAP) by fluorometry as well as several individual antioxidants by high-performance liquid chromatography. As secondary outcome, oxidized genetic damage (7,8-dihydro-8-oxoguanine, known as 8-oxo-Gua) was investigated by the comet assay.

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Both anesthesia techniques (isoflurane or propofol) for a 2-h period resulted in a significant decrease of plasma α -tocopherol, but not other antioxidants including uric acid, carotenoids, and retinol ($P > 0.05$). Propofol, in contrast to isoflurane anesthesia, significantly increased ($P < 0.001$) anti-inflammatory/antioxidant plasma γ -tocopherol concentration in patients. Both anesthesia types significantly enhanced hydrophilic antioxidant capacity and TAP, with no significant difference between them, and 8-oxo-Gua remained unchanged during anesthesia in both groups. In addition, both anesthetics showed antioxidant capacity *in vitro*.

This study shows that anesthesia maintained with either propofol or isoflurane increase both hydrophilic and total antioxidant capacity in plasma, but only propofol anesthesia increases plasma γ -tocopherol concentration. Additionally, both types of anesthetics do not lead to oxidative DNA damage in patients without comorbidities undergoing minimally invasive surgery.

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Abbreviations: 8-oxo-Gua = 7,8-dihydro-8-oxoguanine, AAPH = 2,2'-azobis (4-amidinopropane) dihydrochloride, ASA = American Society of Anesthesiologists, AUC = area under the curve, BODIPY = 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid, CABG = coronary artery bypass graft, CV = coefficient of variation, CYP3A4 = Cytochrome P450 3A4, DCFH = 2',7'-dichlorodihydrofluorescein, DHS = delipidized human serum, DNA = deoxyribonucleic acid, EDTA = ethylenediaminetetraacetic acid, FPG = formamidopyrimidine DNA glycosylase, HEPES = 2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulfonic acid, HO = heme oxygenase, HPLC = high-performance liquid chromatography, IV = intravenous, KCl = potassium chloride, MAC = minimum alveolar concentration, MDA = malondialdehyde, MeO-AMVN = 2,2'-azobis (4-methoxy-2,4-dimethylvaleronitrile), N₂O = nitrous oxide, NaCl = sodium chloride, NaOH = sodium hydroxide, NF κ B = nuclear factor kappa B, OGG1 = 8-hydroxyguanine DNA-glycosylase 1, OR = operating room, PACU = Post-Anesthetic Care Unit, PC = phosphatidylcholine, P_{ET}CO₂ = partial pressure of end-tidal carbon dioxide, ROS = reactive oxygen species, SpO₂ = saturation of peripheral oxygen, TAP = total antioxidant performance.

INTRODUCTION

Among the inhaled anesthetics, isoflurane is a halogenated ether widely used because of its low metabolism rate and low blood–gas partition coefficient. Preconditioning effects of halogenated anesthetics have been suggested to provide cardiac protection in patients undergoing coronary artery bypass graft surgery, which triggers ischemia–reperfusion injury.^{1,2}

However, there are still controversies related to isoflurane-induced oxidative stress.

Propofol is a short-acting intravenous (IV) anesthetic widely employed for induction and maintenance of general anesthesia. Apart from its several anesthetic advantages, it has been reported that this agent exerts a number of nonanesthetic effects.³ The antioxidant capacity of this alkylphenol has been related to its chemical structure, which is similar to that of phenol-based scavengers, such as tocopherol.⁴ Various *in vitro* and animal studies have reported that propofol presents a scavenging activity against a broad range of free radicals and reactive oxygen species (ROS).^{5–7} However, it remains unanswered whether these effects occur in anesthetized patients.

In general, surgical procedures can increase ROS and decrease the biological defense system, leading to oxidative changes. During surgery, activated neutrophils convert oxygen into ROS, such as superoxide radicals, which are dismutated to toxic nonradical hydrogen peroxide, capable of destroying microorganisms. ROS produced by neutrophils can be inhibited by some anesthetics, such as isoflurane,⁸ and may provide a benefit against ischemia–reperfusion injury.⁹

The involvement of ROS in surgical stress has been reported most commonly in major surgeries,¹⁰ but not during minor surgeries without ischemia–reperfusion injury. Moreover, anesthetics used in surgery have demonstrated an important role in the oxidative status of patients. The overproduction of ROS can damage many macromolecules, including deoxyribonucleic acid (DNA). Among such DNA damage, 7,8-dihydro-8-oxoguanine (8-oxo-Gua) is a well-known modification resulting in transversion mutation (GC → TA).¹¹ The intricate network of cellular defense mechanisms includes enzymatic and nonenzymatic antioxidant compounds. Among them, vitamins A and E have shown potent antioxidant activities that ameliorate ROS damage, protecting tissues against oxidative stress.¹²

Taking into account the importance of better understanding the role of anesthetics on oxidative stress in anesthetized patients, the present study was designed to evaluate general anesthesia maintained with isoflurane or propofol on antioxidant status in patients without comorbidities who underwent minimally invasive surgeries. The primary outcomes were to investigate both hydrophilic and total antioxidant capacity as well as several individual antioxidants during different anesthetic techniques. As secondary outcome, 8-oxo-Gua, which is recognized by the 8-hydroxyguanine DNA-glycosylase 1 (OGG1) was investigated.

PATIENTS AND METHODS

Study Design

This study was performed at Sao Paulo State University Hospital (UNESP, Botucatu, Brazil) from 2009 to 2011, was approved by the Institutional Review Board (Human Research Ethics Committee of the Botucatu Medical School-UNESP, #3449–2010), and was registered on the Clinical Trials (NCT00854178).

Fasted adults of both sexes participated in this prospective randomized study. The inclusion criteria consisted of nonobese, nonsmoking, and nonalcoholic adults (18–45 years old) patients classified by the American Society of Anesthesiologists (ASA) as physical status I (healthy patient with no disease other than a surgical abnormality), scheduled for elective minor surgery (septoplasty) lasting at least 2 h. These patients could not be under recent (within 3 months) or current medication or

antioxidant supplementation, radiation or exposure to any environmental pollutant.

An anesthesiologist who was not involved in the perioperative management of the patients printed 30 group identification tags (15 for each group) and placed them in 30 envelopes (1 identification tag per envelope). Then the same anesthesiologist sealed, mixed, and listed the envelopes sequentially, that is, numbered in ascending order. Just before each anesthesia induction, 1 envelope was opened following the sequential order. Thus, the qualified patients were randomly allocated into the following groups: anesthesia maintained with the inhalation isoflurane (isoflurane group) or the IV propofol (propofol group). All participants provided written informed consent before the study. Random allocation, participant inclusion, and intervention distribution were performed by different investigators.

Anesthesia Protocol

In the operating room (OR), a 20-gauge peripheral IV line was inserted in the cephalic vein of the arm. Fluid deficits were replaced with lactated Ringer's solution at 4 mL/kg/h. A Primus anesthesia workstation (Dräger Medical, Lübeck, Germany) was used in all cases. The following standard clinical monitoring was performed: electrocardiogram, saturation of peripheral oxygen (SpO₂), noninvasive arterial pressure (systolic and diastolic), and monitoring of neuromuscular blockade by train-of-four count at the adductor pollicis (TOF-Guard, Organon Teknika/Biometer, Odense, Denmark). The inspiratory and expiratory isoflurane concentrations (isoflurane group), oxygen concentrations, end-tidal carbon dioxide (P_{ET}CO₂), and ventilation parameters were monitored with the Primus built-in monitor. All the patients received active forced-air warming with a specific blanket on the lower limbs using a warming device (Bair Hugger[®], model 750, Arizant Healthcare, Minneapolis, MN).

All patients were premedicated in the OR with IV benzodiazepine midazolam (Dormonid, Roche, Rio de Janeiro, Brazil) (3 mg). In the isoflurane group, anesthesia was induced using the opioid fentanyl (GlaxoSmithKline, Parma, Italy) (5 µg/kg IV) and the hypnotic propofol 2 mg/kg (Diprivan[®], AstraZeneca, Milan, Italy), and was maintained with an end-tidal isoflurane (Abbott, São Paulo, Brazil) at approximately 1.0 minimum alveolar concentration (MAC) equivalent to 1.2%. In the IV group, after the fentanyl (5 µg/kg IV), a target controlled infusion of 1% propofol (Diprivan, AstraZeneca) was administered using a computer-controlled infusion pump (Diprifusor, Fresenius Vial, Brezins, France) programmed with the pharmacokinetic model for propofol.¹³ The initial target propofol plasma concentration was set at 6.0 µg/mL. The target propofol concentrations were maintained between 3.0 and 5.0 µg/mL until the end of surgery. All groups received the neuromuscular blocker rocuronium bromide (Esmeron, Organon, Oss, The Netherlands) (0.6 mg/kg IV). After tracheal intubation, patients' lungs were mechanically ventilated using the volume-controlled mode of the Primus anesthesia workstation 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 to 12 breaths/min to maintain a P_{ET}CO₂ of approximately 35 mm Hg. The intraoperative distal esophageal (core) temperatures were measured after tracheal intubation using a thermocouple sensor (Mon-a-therm 90044[®], Mallinckrodt Medical, Veracruz, Mexico) attached to an electronic thermometer (4070, Mallinckrodt Medical, St Louis, MO). The effectiveness of anesthesia was monitored by assessing the hemodynamic responses (systemic arterial blood pressure

and cardiac rate values within $\pm 20\%$ of baseline). Additional doses of fentanyl ($2 \mu\text{g}/\text{kg}$) and rocuronium ($0.2 \text{ mg}/\text{kg}$) were administered if the patient was considered to be inadequately anesthetized.

Tracheal extubation was accomplished after the complete reversal of the neuromuscular blockade. If necessary, neuromuscular blockade was reversed with neostigmine $30 \mu\text{g}/\text{kg}$ IV (Normastig, União Química, São Paulo, Brazil) and atropine $10 \mu\text{g}/\text{kg}$ IV (Atropion, Ariston, São Paulo, Brazil) at the end of surgery. Patients received dipyrone 1 g IV (Novalgina, Hoechst, Rio de Janeiro, Brazil) and tramadol 100 mg IV (Tramal, Pfizer, São Paulo, Brazil) at the end of surgery. Patients were taken to the Post-Anesthetic Care Unit (PACU) following surgery.

Blood Sampling

Venous blood samples were collected from all patients before (baseline) and 2-h after anesthesia induction, then placed on ice and protected from light. Isolated lymphocytes and plasma were obtained by centrifugation. Aliquots were appropriately frozen at -80°C until analysis. All samples were coded and analyzed blindly. Techniques were carried out under indirect light.

Detection of Lipophilic Antioxidants

Using a reverse phase high-performance liquid chromatography (HPLC) system, the fat-soluble antioxidants—namely carotenoids, retinol (vitamin A), and tocopherols—were measured. Plasma samples were extracted with 2 mL of chloroform:methanol (2:1) followed by 3 mL of hexane. Samples were dried under nitrogen, resuspended into $100 \mu\text{L}$ ethanol, of which $20 \mu\text{L}$ was injected into the HPLC. The HPLC system was a Waters Alliance 2695 (Waters, Wilmington, MA) consisting of a pump with chromatography bound to a 2996 programmable photodiode array detector and a 474 fluorescence detector, a semibore C30 column ($3.0 \times 150 \text{ mm}$), and the software Empower2 Pro. The Waters 2996 programmable photodiode array detector was set at 450 nm for carotenoids, 340 nm for retinoids, and 292 nm for tocopherols. Using this method, lutein, zeaxanthin, cryptoxanthin, carotene, lycopene, retinol, and tocopherol were adequately separated. Carotenoids and retinoids were quantified by determining peak areas in the HPLC chromatograms calibrated against known amounts of standards. The amounts were corrected for extraction and handling losses by monitoring the recovery of the internal standards (echinenone or retinyl acetate). The coefficients of variation (CV) were 4% for inter-assay and 3% for intra-assay. Recovery of internal standard averaged 98%. All sampling was carried out under red light.

Determination of Uric Acid

Hydrophilic antioxidant uric acid was determined by a colorimetric method using a clinical chemistry analyzer (AutoAnalyzer, VITROS 5.1/FS model, Johnson & Johnson, UK), according to the manufacturers' instructions. Data were expressed as $\mu\text{mol}/\text{L}$.

Measurement of Plasma Aqueous Compartment Oxidation

This assay was fluorometrically determined as previously described,¹⁴ with slight modifications. Aqueous plasma oxidizability was initiated by 2,2'-azobis (4-amidinopropane) dihydrochloride (AAPH), used as a peroxy radical initiator whereas 2',7'-dichlorodihydrofluorescein (DCFH) was employed as the marker of oxidative reaction. Briefly, $100 \mu\text{L}$ of plasma, $100 \mu\text{L}$

of phosphate saline, and $100 \mu\text{L}$ of delipidized human serum (DHS) were incorporated and $100 \mu\text{L}$ of DCFH was added and vortexed, and samples were incubated at 37°C for 10 min. AAPH ($500 \mu\text{L}$) was added in each tube with $200 \mu\text{L}$ of phosphate saline. DHS and also samples ($200 \mu\text{L}$) were evaluated in triplicate in a 96-well plate. Oxidation was monitored by the fluorescent oxidation product of DCFH using a microplate reader (Wallac Victor 2, Perkin Elmer Life Sciences, Boston, MA) at 37°C . Samples for each subject and per time point, from both groups, were analyzed within the same run for every assay performed. The experiments were run under red light.

Determination of Total Antioxidant Performance (TAP)

Phosphatidylcholine (PC) liposomes were prepared as previously reported,¹⁵ and TAP was determined following the protocol already described¹⁶ with slight modifications, by using a lipophilic radical initiator 2,2'-azobis(4-methoxy-2,4-dimethylvaleronitrile) (MeO-AMVN) and 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (C11-BODIPY581/591) as a lipophilic fluorescence probe to monitor the lipid compartment plasma oxidation. Plasma ($100 \mu\text{L}$) was added to PC ($100 \mu\text{L}$), which was used as control/standard, and to $300 \mu\text{L}$ of phosphate buffer saline. Samples were incubated at 37°C for 10 min. Then, phosphate buffer ($485 \mu\text{L}$, pH 7.4) was added and gently vortexed. The radical initiator, MeO-AMVN ($15 \mu\text{L}$, final concentration 2 mM), was added slowly to the samples with a syringe, and gently mixed with a magnetic stirring bar. Oxidation was monitored by the green fluorescent oxidation product of BODIPY (final concentration $\lambda_{\text{ex}} = 500$, $\lambda_{\text{em}} = 520 \text{ nm}$, slit 10 nm) using a microplate reader (Wallac Victor 2, Perkin Elmer Life Sciences), at 37°C for 3 h (36 cycles \times 5 min). Wallac workout 2 was used and the following formula was applied: $\text{TAP} = [(\text{AUC control} - \text{AUC plasma})/\text{AUC control}] \times 100$, where AUC = area under the curve; control = PC and plasma sample. The experiments were run under red light.

In order to verify whether the anesthetics could have antioxidant capacity per se, PC liposomes were used for in vitro studies to determine TAP of propofol or isoflurane. Based on patients' plasma concentrations ($3\text{--}5 \mu\text{g}/\text{mL}$), propofol (Diprivan[®]) was added into the solution before the incubation at final concentrations of 1, 3, 5, 7, and $10 \mu\text{g}/\text{mL}$ (mM). For the volatile anesthetic, based on theoretical blood concentrations (MAC and partition coefficient blood:gas; $0.2\text{--}0.8 \text{ mM}$),¹⁷ isoflurane (Abbott) was added into the incubation solution by reverse pipetting on ice at final concentrations of 0.1, 0.3, 0.5, 0.7, and 1.0 mM . The antioxidant α -tocopherol was evaluated as a control (from 5 to 20 mM). All samples were performed and analyzed in triplicate.

Oxidized Purine (8-oxo-Gua) Detected by the Comet Assay

The protocol used followed the procedures previously described,¹⁸ with slight modifications. Every step was carried out under dim light. Volumes of $10 \mu\text{L}$ of lymphocytes were added to $120 \mu\text{L}$ of 0.5% low melting point agarose at 37°C . The mixtures were layered onto slides precoated with 1.5% normal agarose, covered with a coverslip and left for 5 min at 4°C to solidify the agarose. Afterwards, coverslips were carefully removed and slides immersed, overnight, into a cold lysis solution (2.5 M NaCl , 100 mM ethylenediaminetetraacetic acid [EDTA], 10 mM Tris at pH 10, with 1% Triton X-100 and 10%

dimethylsulfoxide added fresh). Slides were washed in phosphate buffer saline for 5 min, washed again (3 × 5 min each) in a buffer 1 × (40 mM 2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulfonic acid [HEPES], 100 mM potassium chloride [KCl], 0.2 mg/mL bovine serum albumin, and 0.5 mM EDTA at pH 8), and incubated at 37°C for 30 min in a moist chamber with 50 µL of OGG1 (1:1000) or 50 µL of enzyme buffer only (control). Then, slides were left for 15 min at 4°C and coverslips were removed. Afterwards, the slides were exposed to a freshly prepared alkaline buffer (1 mM EDTA, 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), slides were fixed in absolute ethanol and stored at 4°C. Slides were stained with 75 µL Sybr Gold and analyzed in a fluorescent microscope at 400× magnification. Images from 100 nucleoids from each treatment/time point/patient were scored using the Comet Assay IV Image System (Perceptive Instruments, Haverhill, Suffolk, UK). Tail intensity was used to estimate the extent of DNA damage.

Statistical Analysis

Sample size was calculated from a pilot study and also from previous studies^{19,20} based on antioxidant level. Fifteen patients per group allowed a 95% statistical power (type II error – β of 0.05) to detect a between-group difference of 3.4 µM in γ-tocopherol concentrations (SD of ±2.6 µM) with an α error of 0.05. The characteristics of the 2 groups were compared by Student *t* test. The repeated measures ANOVA followed by Tukey’s test were used to compare time points and groups for all

measurements. For all the analyses, a *P* value <0.05 was considered statistically significant.

RESULTS

The flow diagram of the study procedure is shown in Figure 1. Characteristics of the studied population are shown in Table 1. None of the patients presented any surgical or clinical complications, and all of them were discharged from the hospital according to the established guidelines. Patients from both groups were hemodynamically stable (data not shown).

Individual Antioxidants

All the carotenoids and retinol evaluated slightly decreased during 2 h of anesthesia, but without statistical significance, and with no difference between the groups (Table 2). Both types of anesthesia significantly decreased α-tocopherol, but there was no statistically difference between groups. Isoflurane or propofol anesthesia did not alter the uric acid concentrations in plasma (Table 3).

Patients from the 2 groups presented similar levels of γ-tocopherol before anesthesia (baseline), but they differed at 2-h after anesthesia induction (*P* = 0.02). Plasma γ-tocopherol concentration significantly increased with propofol (1.6× fold) (*P* < 0.001), but remained unchanged during isoflurane anesthesia (Figure 2).

Antioxidant Capacity

Both isoflurane and propofol anesthesia significantly increased the antioxidant capacity in the aqueous compartment

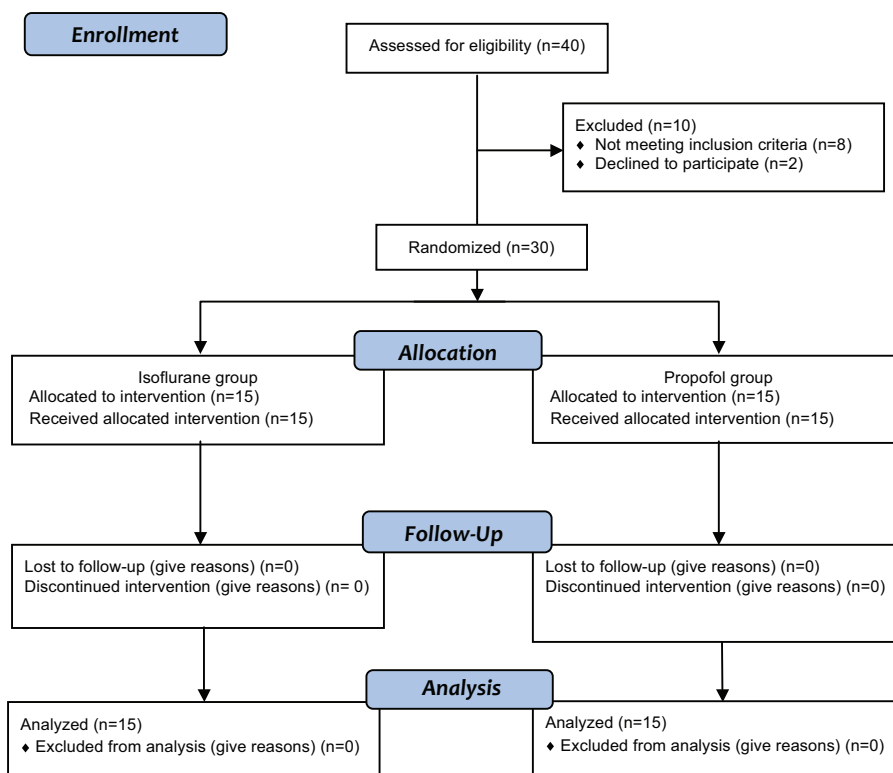


FIGURE 1. Flow diagram of the study.

TABLE 1. Characteristics of the Studied Populations

Characteristics	Groups		P
	Isoflurane (n = 15)	Propofol (n = 15)	
Age, yr	25.1 ± 7.3	28.8 ± 9.4	0.22
Weight, kg	67.9 ± 12.5	68.7 ± 11.1	0.86
Height, cm	167.4 ± 8.4	170.6 ± 10.8	0.35
Intraoperative anesthesia			
Fentanyl, µg	473.8 ± 139.4	517.2 ± 111.3	0.34
Rocuronium bromide, mg	44.3 ± 6.6	51.9 ± 13.6	0.10

Data are expressed as means (X) ± standard deviation (SD).

of plasma ($P < 0.05$) as well as TAP ($P < 0.05$) evaluated in patients during surgery (Figures 3 and 4, respectively). An enhancement of antioxidant status at 2-h of anesthesia was observed in patients anesthetized with isoflurane or propofol, without any significant differences between the groups.

Figure 5 shows the oxidation kinetics of PC liposomes in the presence of either isoflurane or propofol, as determined by TAP. Both anesthetics presented similar responses and showed dose-dependent antioxidant potential in the equivalent blood concentration during anesthesia. Figure 6 shows the antioxidant protection of both anesthetics at different concentrations, including those utilized for anesthesia maintenance. Both anesthetics showed antioxidant capacity (around 11% and 14% for isoflurane and propofol, respectively).

Oxidative DNA Damage

General anesthesia maintained with isoflurane or propofol did not induce 8-oxo-Gua damage in lymphocytes of patients

2 h after anesthesia induction as measured by the comet assay, when specific glycosylase OGG1 was used (Table 4). Neither the time points nor groups differed significantly ($P > 0.05$).

DISCUSSION

In this randomized and prospective clinical trial study, we showed the antioxidative role of propofol and isoflurane anesthesia in patients without comorbidities undergoing minimally invasive surgery without ischemia–reperfusion injury. Antioxidant activity of isoflurane itself may have resulted in increases of plasma antioxidant capacity in adult patients whereas propofol may have contributed to the increase of γ -tocopherol and also antioxidative status in these patients. Both anesthetics seem to prevent systemic oxidative DNA damage.

All the nonenzymatic antioxidants determined in the present study are able to quench the peroxy radical, which is involved in lipid peroxidation. Despite the slight decline of some antioxidant concentrations (but still at normal ranges), neither isoflurane nor propofol anesthesia had any effect on malondialdehyde (MDA) values, as demonstrated in our previous study.²¹ Isoflurane plus nitrous oxide (N₂O) anesthesia had no effect on the plasma antioxidant enzymatic system, but decreased some erythrocyte enzyme activities and trace elements in patients undergoing abdominal surgery.²² In this same type of surgery, patients under total IV propofol anesthesia showed enhancement of antioxidant glutathione-peroxidase measured in platelets.²³

We believe one of the novelties observed in the present study is the increase of γ -tocopherol in patients anesthetized with propofol, but not under isoflurane, in minor elective surgery, since humans cannot synthesize tocopherol, and patients were fasted and not under supplementation. The γ -tocopherol is the major form of vitamin E in many plant seeds and vegetable oils.²⁴ Since propofol is usually eluted in an

TABLE 2. Micronutrients (µM) Detected in Patients Undergoing Surgery Maintained With Isoflurane (n = 15) or Propofol (n = 15) Anesthesia

Micronutrients	Anesthetic Groups	Time Points		P
		Baseline	2-h After Anesthesia Induction	
Luthein	Isoflurane	0.19 ± 0.13	0.16 ± 0.11	0.88
	Propofol	0.15 ± 0.04	0.13 ± 0.05	0.10
Zeaxanthin	Isoflurane	0.08 ± 0.05	0.07 ± 0.04	0.86
	Propofol	0.06 ± 0.03	0.05 ± 0.02	0.88
Cryptoxanthin	Isoflurane	0.12 ± 0.17	0.10 ± 0.14	0.97
	Propofol	0.11 ± 0.06	0.10 ± 0.05	0.98
α-carotene	Isoflurane	0.10 ± 0.09	0.09 ± 0.08	0.99
	Propofol	0.11 ± 0.08	0.09 ± 0.07	0.95
β-carotene	Isoflurane	0.23 ± 0.13	0.20 ± 0.11	0.98
	Propofol	0.27 ± 0.25	0.23 ± 0.20	0.93
Lycopene	Isoflurane	0.35 ± 0.14	0.30 ± 0.12	0.13
	Propofol	0.42 ± 0.20	0.35 ± 0.16	0.51
Cis-lycopene	Isoflurane	0.26 ± 0.11	0.23 ± 0.09	0.96
	Propofol	0.33 ± 0.19	0.28 ± 0.16	0.78
α-tocopherol	Isoflurane	24.86 ± 11.83	21.44 ± 12.00	0.002
	Propofol	24.69 ± 11.80	21.54 ± 9.19	<0.001
Retinol	Isoflurane	2.21 ± 0.64	1.85 ± 0.63	0.35
	Propofol	2.23 ± 0.53	1.86 ± 0.42	0.35

Data are expressed as means (X) ± standard deviation (SD). $P > 0.05$ between groups at both time points.

TABLE 3. Uric Acid Levels (μM) in Patients Undergoing Surgery Maintained With Isoflurane ($n=15$) or Propofol ($n=15$) Anesthesia

Groups	Time Points		P
	Baseline	2-h After Anesthesia Induction	
Isoflurane	299.5 \pm 71.7	285.5 \pm 68.4	0.97
Propofol	300.8 \pm 91.4	266.4 \pm 88.6	0.68

Data are expressed as means (X) \pm standard deviation (SD). $P > 0.05$ between groups at both time points.

intralipid solution (Diprivan[®]) containing soybean oil with a mixture of saturated and unsaturated fatty acids, which are rich in γ -tocopherol, this could explain our findings. The increase of γ -tocopherol concentration only in the propofol group could also be attributed to the interaction of this anesthetic with Cytochrome P450 3A4 (CYP3A4), since the anesthetic and tocopherols share this metabolic pathway.¹⁹

Drawing a parallel, a 6-week γ -tocopherol supplementation results in high concentrations of this antioxidant (similarly to our observations in the propofol group) thus reducing biomarkers of oxidative stress and inflammation in metabolic syndrome patients.²⁵ A study reported that propofol anesthesia (plasma concentrations of 2.5–4.0 $\mu\text{g/mL}$) enhances γ -tocopherol during cardiac surgery with cardiopulmonary bypass, returning to basal values 24 h after surgery.¹⁹ These findings indicate an anti-inflammatory effect of propofol, which may be relevant for controlling inflammatory response related to tissue injury after reperfusion.

Plasma γ -tocopherol was increased during propofol anesthesia whereas α -tocopherol was decreased during continuous infusion with propofol or even with isoflurane. Previous studies reported that antioxidant supplementation leads to a negative correlation between α - and γ -tocopherol.^{26,27} Another report supports our data in isoflurane group since the authors showed a decrease of vitamin E 1 h after the introduction of this volatile anesthetic in patients who underwent cholecystectomy.²⁸ In contrast, this same antioxidant (α -tocopherol)

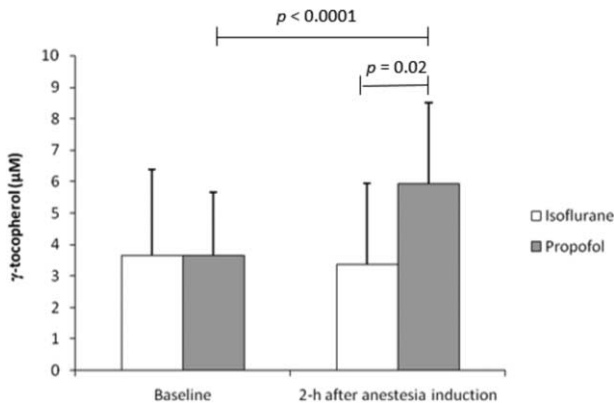


FIGURE 2. Changes in γ -tocopherol detected in patients undergoing surgery maintained with isoflurane ($n=15$) or propofol ($n=15$) anesthesia. $P > 0.05$ between groups at baseline.

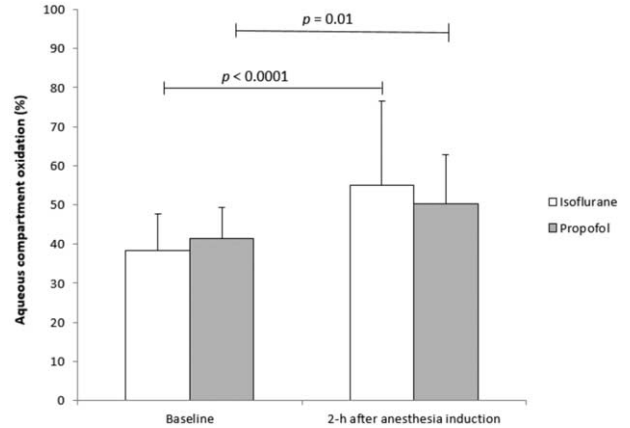


FIGURE 3. Hydrophilic antioxidant capacity evaluated in patients undergoing surgery maintained with isoflurane ($n=15$) or propofol ($n=15$) anesthesia. $P > 0.05$ between groups at both time points.

remained unchanged during and after propofol or isoflurane anesthesia in patients scheduled for lumbar spinal surgery.²⁹ Possible discrepancies in these data could be explained by different anesthetic regimens, including the use of isoflurane (0.5% to 0.75%) together with N_2O (60%), and different plasma concentrations of propofol.

Compared with α -tocopherol, γ -tocopherol is a slightly less potent antioxidant as an electron donor despite being much superior in detoxifying electrophiles, including peroxynitrite, and presents an anti-inflammatory effect. Interestingly, an inflammatory response occurred earlier in patients who received isoflurane compared with propofol anesthesia.³⁰ Moreover, like propofol, γ -tocopherol reduces inflammation through inhibition of cyclooxygenase type 2 activity.³¹

Most of previous studies using propofol and derivatives were performed under aqueous (nonmembranous) conditions in vitro.^{32,33} Thus, our findings indicate that propofol administered by a computer-controlled infusion pump is able to increase antioxidant capacity when evaluated in the hydrophilic compartment of the patient's plasma.

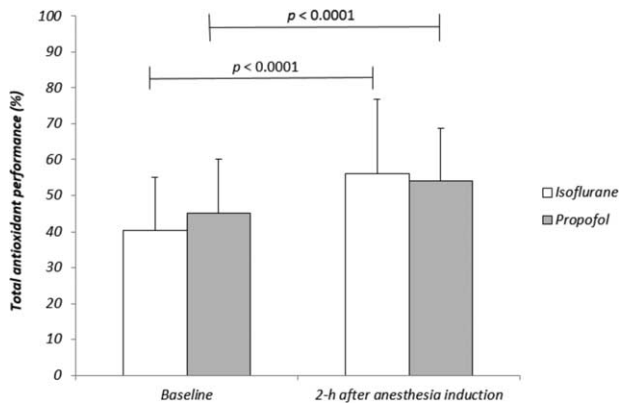


FIGURE 4. Total antioxidant performance evaluated in patients undergoing surgery maintained with isoflurane ($n=15$) or propofol ($n=15$) anesthesia. $P > 0.05$ between groups at both time points.

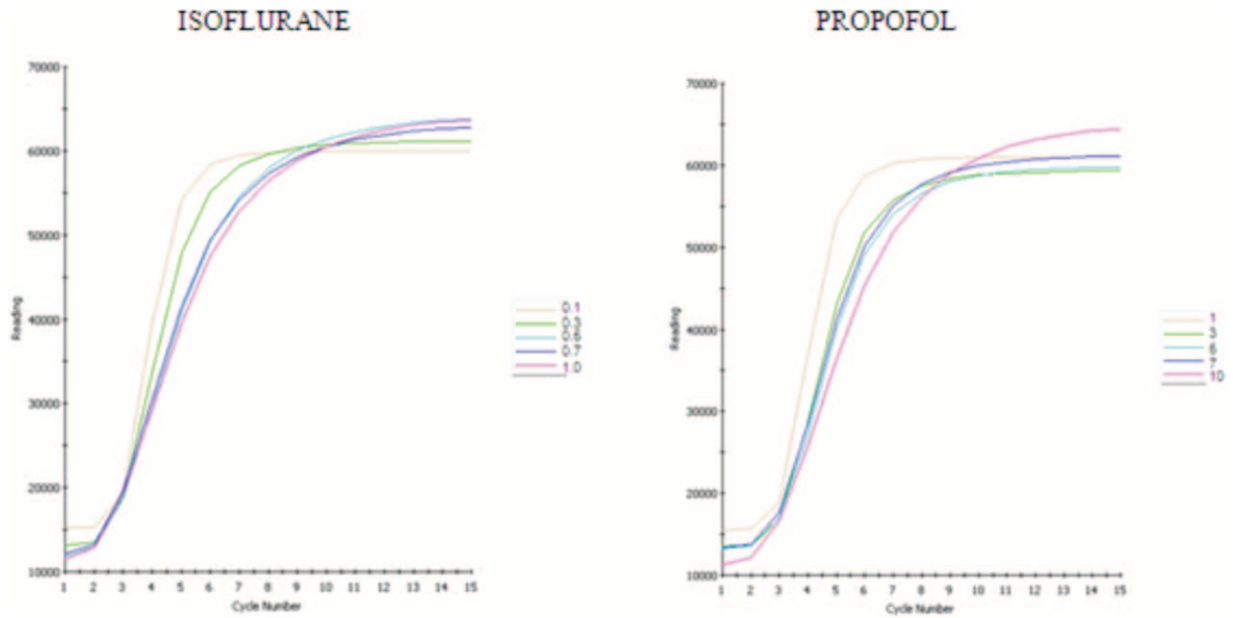


FIGURE 5. Oxidation kinetics of total antioxidant performance assay (fluorescence) with different concentrations of isoflurane or propofol. Each cycle corresponds to 5 min.

Propofol has a great affinity for lipid bilayers, being preferentially distributed in liposomal and cellular membranes, accounting for its much higher concentration in membranes than in blood. This anesthetic reacts with peroxynitrite, leading to formation of a propofol-derived phenoxyl radical, and acts

similarly to tocopherol.^{4,34} The majority of in vitro and in vivo studies have attributed the protective effect of bioactive polyphenols to their chemical reactivity toward free radicals and their capacity to prevent oxidation of important intracellular components. This anesthetic displays antioxidant capacity and inhibits lipid peroxidation at low μM levels, which correspond to the human plasma concentration during anesthesia.⁷ Moreover, propofol showed great potential as a free radical scavenger in patients undergoing surgery with extracorporeal circulation.³⁵

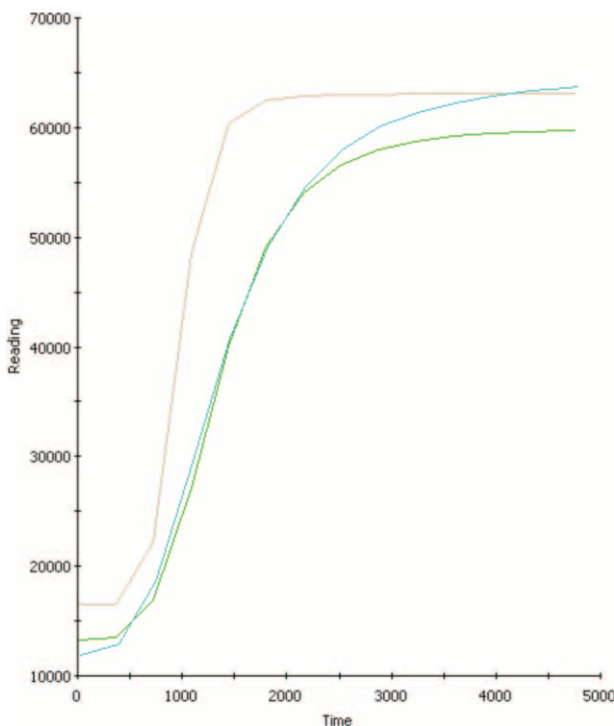


FIGURE 6. Total antioxidant performance of control (phosphatidylcholine—brown line), isoflurane (blue line), or propofol (green line), at blood concentrations.

In order to assess the magnitude of the active interactions between the 2 compartments in plasma, we used the fluorometric method denominated TAP to determine the total antioxidant effects of patients undergoing anesthesia. TAP measures both aqueous and lipid compartment oxidizability.¹⁴ Thus, our data showed that both propofol and isoflurane are able to augment both the aqueous compartment as well as the hydrophilic and lipophilic antioxidant capacity.

It has been suggested that polyphenols induce the heme oxygenase (HO)-1 pathway.³⁶ In the same manner, it has already been reported that anesthetics also modulate HO, which exerts anti-inflammatory, anti-proliferative, antioxidative, and vasodilatory effects.³⁷ Therefore, both propofol and isoflurane share similar mechanisms of HO-1 induction via the nuclear factor kappa B.³⁸ Our findings reinforce the notion that patients anesthetized with propofol or isoflurane have increased antioxidant capacity, even when administered in minor surgeries without ischemia–reperfusion injury.

To elucidate whether the increase of hydrophilic antioxidant capacity and TAP in patients undergoing an anesthetic procedure could be a consequence of antioxidant capacities from anesthetics, the oxidation kinetics of either propofol or isoflurane were determined in vitro. Anesthetics by themselves showed antioxidant capacities, reinforcing our findings related to the increase of antioxidant status in anesthetized patients. Thus, isoflurane may have contributed to enhanced antioxidant status in patients even during a relatively short

TABLE 4. Oxidative DNA Damage (Using Lesion-Specific-Endonuclease) Evaluated in Patients Undergoing Surgery Maintained With Isoflurane (n = 15) or Propofol (n = 15) Anesthesia

DNA Damage, %	Anesthetic Groups	Time Points		P
		Baseline	2-h After Anesthesia Induction	
Oxidative damage (OGG1)	Isoflurane	18.2 ± 7.5	16.6 ± 9.7	0.82
	Propofol	17.4 ± 8.9	14.0 ± 8.4	0.67

Data are expressed as means (X) ± standard deviation (SD). $P > 0.05$ between groups at both time points. DNA = deoxyribonucleic acid

surgical-procedure duration. This volatile anesthetic is being utilized especially in major surgeries, including coronary ones, related to ischemia–reperfusion injury. Moreover, this anesthetic can attenuate oxidative stress and has neuroprotective effects in vitro, and its pretreatment protects cardiac cells from damage by oxidative stress and reduces myocardial infarction size by modulating mitochondrial ROS at clinical concentrations.^{39–41}

Additionally, there was no oxidative genetic-induced lesion, evaluated by the use of OGG1 in the modified comet assay, during isoflurane anesthesia. There are controversial findings related to the cellular toxicity of volatile anesthetics. Isoflurane might induce cytotoxicity in vitro,⁴² but anesthesia maintained with isoflurane did not lead to genotoxicity or apoptotic T lymphocytes in patients.⁴³ It must be highlighted that only 0.2% of isoflurane undergoes metabolism, and does not alter the extent of DNA damage induced by in vitro treatment with hydrogen peroxide.⁴³ Hence, these data support the hypothesis that 1.2% isoflurane does not induce oxidative lesions in patients without comorbidities undergoing minor elective surgeries.

In relation to genetic damage, propofol prevented DNA cleavage in peroxynitrite injury in primary astroglial cell cultures.⁶ Although we have already observed a reduction of genetic lesions evaluated by formamidopyrimidine DNA glycosylase (FPG) sites during propofol anesthesia,⁴⁴ we did not detect a decrease in oxidized DNA using OGG1 when total IV anesthesia with propofol was administered. This could be explained by different types of oxidative damage recognized by OGG1 or FPG repair enzyme. The OGG1 is more specific for detecting methyl-fapy-guanine and 8-oxo-Gua, which is the most abundant metabolite of guanine and seems to play a major role in mutagenesis and in carcinogenesis.^{18,45} In a previous study, we reported a decrease of hydrogen peroxide-induced genotoxicity in lymphocytes as well as a reduction of apoptotic T helper cells collected from patients during surgical procedures with propofol anesthesia.⁴⁴

One limitation of our study is that we have used propofol for anesthesia induction in isoflurane group, but since this IV anesthetic was only administered in a single dose, and considering its high clearance,⁴⁶ it seems to have had only a minimum influence on oxidative status during surgery.

This is the first study to show that both types of anesthesia increase antioxidant status and do not lead to oxidized (8-oxo-Gua) genetic damage in adult patients during surgical procedure. Additionally, propofol anesthesia contributes to enhance the anti-inflammatory/antioxidant plasma γ -tocopherol concentration in these patients. Consequently, the clinical application of this study is very important. We

recommend the anesthesiologists and surgeons to apply this knowledge into routine clinical practice. These findings can provide support for the translational concept by further elucidating the antioxidative role of 2 anesthetics widely used in daily practice during surgeries. Further studies are also required to better understand the possible action mechanisms of such anesthetics related to antioxidant capacity in patients undergoing major surgeries or with comorbidities.

In conclusion, the present study shows that anesthesia maintained with either propofol or isoflurane increase both hydrophilic and total antioxidant capacity in plasma, but only propofol anesthesia increases plasma γ -tocopherol concentration. In addition, both types of anesthetics do not lead to oxidative DNA damage in patients without comorbidities undergoing minimally invasive surgery.

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