Genotoxicity, cytotoxicity and gene expression in patients undergoing elective surgery under isoflurane anaesthesia

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There are numerous studies reporting on the effects of inhalation anaesthesia in cells of exposed individuals but not much is known about the ability of isoflurane (ISF) to induce oxidative DNA damage. However, surgery is often associated with a temporary perioperative immunological alteration, and some volatile anaesthetics seem to contribute to a transient lymphocytopenia after surgery. We conducted a study to evaluate a possible genotoxic effect, including oxidative DNA damage, and apoptosis in peripheral lymphocytes of 20 patients American Society of Anaesthesiologists physical status I undergoing minor elective surgery lasting at least 120 min, under anaesthesia with ISF. We also investigated the expression of several genes in blood cells. Blood samples were collected at three time points: before anaesthesia (T1), 2 h after the beginning of anaesthesia (T2) and on the first post-operative day (T3). General DNA damage and oxidised bases (Fpg and endo III-sites) in blood lymphocytes were evaluated using the comet assay. Lymphocytes were phenotyped and apoptosis was evaluated by flow cytometry. In addition, expressions of hOGG1 and XRCC1, genes involved in DNA repair, and BCL2, a gene related to apoptosis, were assessed by quantitative real-time polymerase chain reaction. Results showed no statistically significant difference in the level of DNA damage and oxidised bases among the three sampling times. Anaesthesia with ISF did not increase the percentage of cells in early or late apoptosis in cytotoxic or helper T lymphocytes. Lower hOGG1 and BCL2 expressions were detected at T3 in comparison to the other two previous time points, and there was significantly lower expression of XRCC1 at T3 in relation to T2. In conclusion, the exposure to ISF did not result in genotoxicity and cytotoxicity in lymphocytes and in toxicogenomic effect in leucocytes, although DNA repair and apoptosis-related genes were down-regulated on the first post-operative day.

Introduction

DNA is continuously exposed to a variety of biological, chemical and physical agents, which may alter its structure, modifying its function (1). Among exogenous compounds, anaesthetic gases, commonly used in general anaesthesia procedures, have attracted attention because of concern about their potential for having genotoxic effects (2–4). Worldwide, ~100 million people every year undergo surgery, with the majority of patients receiving inhalation general anaesthesia (5). Isoflurane (ISF) and sevoflurane are the most widely used inhaled halogenated anaesthetics. Due to its low metabolism rate and low blood-gas partition coefficient, which decreases its induction and recovery times, the introduction of ISF [2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane] in clinical practice represented a significant advance for inhalation anaesthesia (6). It is well known that oxidative DNA damage can be repaired, especially by the base excision repair system, which include these same authors believe that anaesthetic genotoxicity might also be due to their metabolic oxidation or reduction, giving rise to reactive metabolites and reactive oxygen species (ROS). It is well known that oxidative DNA damage can be repaired, especially by the base excision repair system, which include hOGG1 (human 8-oxoguanine DNA glycosylase 1) and XRCC1 (X-ray cross-complementing group 1 protein) (19,20). However, it is not known about the interference of anaesthetics on the expressions of such genes.

Evidence of immune system dysfunctions has been frequently reported after surgery, which can lead to profound, but transient, depletion of all types of lymphocytes (21). Although the mechanism underlying the decrease of the number of immunological cells is still not clear, it may result from lymphocytes being induced to enter into apoptosis (22). It has been reported that halogenated anaesthetics in vitro cause lymphocytopenia due to apoptosis via up-regulation of caspase-3 (23). Similarly, elevated rates of apoptosis 24 h after invasive surgery under ISF or nitrous oxide (N2O)
anaemia have been observed in cultured T lymphocytes (24,25). In contrast, it has been reported that ISF protected cardiomyocytes and reduced the level of apoptosis induced by hydrogen peroxide and hypoxia in rodents (26).

In the literature, there is no information about the ISF genotoxicity in healthy young patients undergoing non-invasive surgeries. Furthermore, it remains controversial the interference of possible confounding factors, such as age, physical status of the patients and type of surgery on the genotoxic potential of the anaesthetic. Therefore, the present study aimed to evaluate general DNA damage and specifically oxidised purines and pyrimidines induced by ISF in lymphocytes of patients undergoing minor surgeries. Additionally, it was also investigated whether the exposure to the anaesthetic could interfere with the immune system and also with the expression of DNA repair (hOGG1 and XRCC1) and apoptosis (BCL2)-related genes.

Materials and methods

Subjects

The Ethical Committee for Human Research from Botucatu Medical School—Universidade Estadual Paulista (581/2006—Botucatu, São Paulo, Brazil) approved the protocol used in the present study. After signing the informed consent, all the patients answered a detailed questionnaire about their lifestyle, health status and previous exposure to environmental pollutants. Patients with a disease, smokers, alcoholics and those who were under medication or antioxidant supplement or who had received radiation were excluded. Twelve male and eight female non-smoker adults classified by the American Society of Anaesthesiologists (ASA) as physical status I patients (healthy patient, with no disease other than surgical abnormality and with no systemic disturbances) with normal body mass index (23.5 ± 3.5 kg/m²), aged from 18 to 45 years (25.1 ± 6.8 years) and scheduled for elective minor otorhinolaryngology surgery lasting at least 120 min (106.4 ± 55.8 min), at Botucatu Medical School Hospital, were enrolled in this study. Propofol (160 ± 46.7 mg), fentanyl (462 ± 151.3 μg) and rocuronium (44.4 ± 7.1 mg) were used during anaesthesia.

General anaesthesia procedure

Standard clinical monitoring was performed: electrocardiogram, peripheral oxygen saturation (SpO₂), non-invasive arterial pressure (systolic and diastolic), end-tidal CO₂ (PetCO₂) and ISF and monitoring of neuromuscular blockade. All patients were premedicated in the operating room with intravenous (IV) benzodiazepine midazolam (3 mg; Roche, São Paulo, Brazil). Anaesthesia was induced with opioid fentanyl (5 μg/kg IV; Janssen, São Paulo, Brazil), hypnotic agent propofol (2 mg/kg IV; Astra Zeneca, Milan, Italy) and rocuronium bromide (0.6 mg/kg IV; Organon, Oss, Holland), which is a neuromuscular blocker that was given to facilitate orotracheal intubation. The lungs were mechanically ventilated with 40% oxygen (0.8 l/min) in air (1.2 l/min). ISF (bromide (0.6 mg/kg IV; Organon, Oss, Holland), which is a neuromuscular agent propofol (2 mg/kg IV; Astra Zeneca, Milan, Italy) and rocuronium (0.2 mg/kg IV; Ariston, São Paulo, Brazil) were used when necessary, if the patients needed additional doses of fentanyl (2 μg/kg IV; Janssen, São Paulo, Brazil), fentanyl (462 ± 137.4 mg), fentanyl (462 ± 76.4 mg), fentanyl (462 ± 15.7 mg) and rocuronium (44.4 ± 7.1 mg) were used during anaesthesia. Anaesthesia was assessed by haemodynamic responses, and additional doses of fentanyl (2 μg/kg IV; Janssen, São Paulo, Brazil) were utilised for antiemesis. Dipyrone (1 g; Hoechst, Rio de Janeiro, Brazil) was used for post-operative analgesia, at the end of the surgery. If necessary, additional doses of fentanyl (2 μg/kg IV; Janssen, São Paulo, Brazil) and dipyrone (1 g; Hoechst, Rio de Janeiro, Brazil) were used during anaesthesia. Anaesthesia was assessed by haemodynamic responses, and additional doses of fentanyl (2 μg/kg IV; Janssen, São Paulo, Brazil), fentanyl (462 ± 137.4 mg), fentanyl (462 ± 76.4 mg), fentanyl (462 ± 15.7 mg) and rocuronium (44.4 ± 7.1 mg) were used during anaesthesia.

Blood sampling

Venous blood samples from all patients undergoing inhalation anaesthesia were drawn at three time points: before premedication and anaesthesia (T₀), 2 h after the beginning of anaesthesia (T₂) and on the first post-operative day (T₃). Blood was collected in sodium heparin tubes (10 ml) for immediate lymphocytes isolation and in PAXgene Blood RNA Tubes (Qiagen/PreAnalytiX, Hombrechtkon, Switzerland) for RNA stabilisation. These tubes were kept at room temperature (RT) for 12 h and then placed into a freezer maintained at −20°C until RNA isolation.

Chemicals

Ethidium bromide, HEPES and bovine serum albumin were purchased from Sigma (St Louis, MO, USA); normal and low melting point agaroses, EDTA and Tris from Invitrogen (Carlsbad, CA, USA); hydrogen peroxide (H₂O₂), H₂BO₃, NaCl, NaOH, KCl, HCl, NaHCO₃, KH₂PO₄ and Na₂HPO₄ from Merck (Germany); Ficoll–Paque® from GE (Sweden); dimethylsulfoxide from Malinkirk (Mexico); Triton X-100 from J. T. Baker (Phillipsburg, NJ, USA); annexin labelled with fluorescein isothiocyanate (FITC) and annexin V buffer, 7-amino-actinomycin D (7-AAD), phycoerythrin (PE)-labelled monoclonal antibodies anti-hCD4 and anti-hCD8 were purchased from Becton Dickinson (San Jose, CA, USA) and endonuclease III (endo III) and formamidopyrimidine DNA glycosylase (Fpg) from New England Biolabs (Ipswich, MA, USA).

Lymphocyte isolation

Lymphocytes were isolated in Ficoll–Paque® gradients. Samples of peripheral blood (2 ml) were mixed with 2 ml of phosphate-buffered saline (PBS), layered over 3 ml of Ficoll and centrifuged at 1100 × g for 30 min, at RT. The lymphocyte layer was removed, mixed with 4 ml PBS, centrifuged at 400 × g for 15 min and the cell pellet was resuspended in PBS (27). Lymphocytes were used for comet assay and flow cytometry.

Alkaline comet assay

The protocol used followed the general procedures used by Singh et al. (28) and Tice et al. (29), with some modifications. Every step was carried out under indirect light. Slides were coded and blindly analysed. Volumes of 10 μl of fresh lymphocytes (~1 × 10⁶ cells/μl) were added to 120 μl 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, the coverslips were carefully removed and the slides immersed, overnight, into a cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris at pH 10, with 1% Triton X-100 and 0.1% dimethylsulfoxide added fresh). To evaluate oxidative DNA damage, slides were washed in PBS for 5 min and washed again (3 × 5 min each) in a buffer × 1 (40 mM HEPES, 100 mM KCl, 0.2 mg/ml bovine serum albumin and 0.5 mM EDTA at pH 8). Slides were incubated at 37°C for 30 min in a moist and dry chamber, with 100 μl of Fpg and endo III (1:1000) that recognises oxidative DNA damage and single-strand breaks, respectively, at with 100 μl of enzyme buffer only (control). Subsequently, the slides were left for 15 min at 4°C and then the 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 0.8 V/cm and 300 mA for 30 min. Following 15 min neutralisation with 0.4 M Tris (pH 7.5), the slides were fixed in absolute ethanol and then stored at 4°C. Lymphocytes sampled at the three time points from exposed patients were also treated with hydrogen peroxide (H₂O₂) at 100 μM for 30 min at 4°C to evaluate the sensitivity of these cells to oxidative DNA damage.

The slides were stained with 50 μl ethidium bromide (20 μg/ml) and analysed in a fluorescent microscope at ×40 magnification. Images from 100 nuclei were captured in two replicates, for each treatment/time point/patient. Nucleoids (two replicates) per each treatment/time point/patient were scored and analysed in a fluorescent microscope at ×200 magnification. Tail intensity (%DNA in tail) was used to estimate the extent of DNA damage.

Phenotyping and apoptosis detection

T-lymphocytes phenotyping and assessment of apoptosis were performed in a FACSCalibur Flow Cytometer (BD Biosciences). The percentage of cells in early apoptosis was quantified using the annexin V-FITC method, which detects phosphatidylserine translocated from the inner cell membrane (annexin-V⁺/7-AAD⁻); cells in late apoptosis were quantified based on the presence of annexin-V⁻/7-AAD⁺ and viable cells were also analysed (annexin-V⁻/7-AAD⁻). The annexin-V is an important marker of early apoptosis because it happens before DNA fragmentation (30).

Freshly obtained lymphocytes were distributed into aliquots (~1 × 10⁶ cells/ml) and individually incubated: one tube was mixed with isoton, a saline solution with no antibodies (auto-fluorescence), vortexed and incubated at RT, in the dark, for 15 min; other aliquots, following PE-labelled monoclonal antibodies anti-CD4 and anti-CD8, were phenotyped to differentiate CD4⁺ T cells and CD8⁺ T cells. Lymphocytes were mixed with 100 μl of annexin V-binding buffer (diluted 1:10 with distilled water), 5 μl of annexin V-FITC, 5 μl of 7-AAD and 10 μl of anti-CD4 or anti-CD8, vortexed and incubated for 15 min at RT in the dark. Afterwards, 400 μl aliquot of annexin V-binding buffer × 1 was added to each tube, samples were vortexed and the flow cytometry analysis was performed. Gating on lymphocytes (10 000 events were acquired.
for each sample) by forward and side scatter (cell size versus granularity) for defining the target population. Data were analysed by the Cell Quest and FACSComp Programs.

Analysis of hOGG1, XRCC1 and BCL-2 mRNA by quantitative real-time polymerase chain reaction

RNA was isolated with the PAXgene Blood RNA Kit according to the manufacturer’s protocol (Qiagen/PreAnalytiX). The concentration of total RNA was determined by spectrophotometer, and each sample was assessed for purity by absorbance (A260/A280 nm between 1.9 and 2.1). Samples integrity was assessed by 1.5% agarose gel using Tris/borate/EDTA buffer. RNA was reverse-transcribed complementary DNA (cDNA) with a High Capacity cDNA Reverse Transcription Kit according to the manufacturer’s protocol (Applied Biosystems—ABI, Foster City, CA, USA). The reactions were firstly incubated at 25°C for 10 min and 37°C for 120 min and then at 4°C. Samples were placed into a freezer at −20°C until polymerase chain reaction (PCR).

Quantitative real-time PCR was performed using Taqman FAM–MGB probes and primers, ordered as inventoried from ABI for the genes hOGG1 (assay ID Hs00213454_m1), XRCC1 (assay ID Hs00959834_m1) and BCL2 (assay ID Hs00608023_m1). After comparing candidate genes for the endogenous control, β-actin (ACTB) was selected for this study. For amplification, TaqMan Universal PCR Master mix (ABI) was used and a final of 10 μl volume was used for each reaction. Thermal cycling and real-time detection of the fluorescence were carried out in an ABI Prism 7500 FAST using the following amplification parameters: denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec. Negative control (no cDNA) in duplicate was added in each plate to ensure no contamination. For the control, a pool of cDNA sample from healthy subjects was used. All samples were run simultaneously with the test samples throughout the experiments. Cycle threshold (Ct) values were determined as the cycle where ROX-normalised fluorescence over background was significantly above background levels. Fold induction was calculated using the formula $2^{-\Delta\Delta Ct}$ (31). In the pool samples (control), relative quantification (RQ) for hOGG1 varied from 0.8 to 1.2; for XRCC1 from 0.8 to 1.3 and for BCL2 from 0.7 to 1.1. Based on these data, genes were considered up-regulated when RQ from patients were $\geq$1.5; down-regulation was considered when RQ $<0.5$.

Statistical analysis

The non-parametric Friedman test was used to compare the extent of DNA damage, the frequency of viable cells and apoptosis and the extent of gene expression (data expressed as median and first and third quartiles) at the three blood sampling times. When two variables were compared, the Mann–Whitney test was performed. Values with $P < 0.05$ were considered statistically significant.

Results

Inhalation anaesthesia with ISF was not genotoxic

General anaesthesia with the ISF at 1.0 minimum alveolar concentration did not induce DNA single- and double-strand breaks and alkali-labile sites in lymphocytes, as measured by the comet assay, when evaluated 2 h after the beginning of anaesthesia (T2) or on the first post-operative day (T3). Similar results ($T_1 = T_2 = T_3$) were found when Fpg and endo III were used to detect oxidised purines and pyrimidines, respectively (Table I).

Table II shows the results of DNA damage in lymphocytes sampled at the three time points, when challenged in vitro with H2O2. Significant increase of damage ($P < 0.05$) was observed in $T_1$, $T_2$ and $T_3$, when compared to the control (without treatment), but with no statistical difference among the three sampling times.

General anaesthesia did not induce apoptosis in T lymphocytes

The percentage of viable, early and late apoptotic helper T (CD4+) and cytotoxic T cells (CD8+) from all the patients indicated that inhalation anaesthesia with ISF was not cytotoxic in lymphocytes (Table III). No statistically significant ($P > 0.05$) difference in the frequency of early apoptosis annexin-V+/7-AAD was detected among the three sampling times ($T_1$, $T_2$ and $T_3$) in both subtypes of T lymphocytes.

Quantitative real-time PCR

Expression of hOGG1 and XRCC1 in peripheral leukocytes from patients undergoing general anaesthesia with ISF is showed in Figures 1 and 2, respectively. Significant down-regulation of hOGG1 was observed on the first day after surgery ($T_3$), when compared to $T_1$ and $T_2$. XRCC1 also showed down-regulation at $T_3$ ($P < 0.05$) but only in relation to $T_2$. Similarly to hOGG1, the anti-apoptotic gene BCL2 was down-regulated ($P < 0.05$) on the first post-operative day ($T_3$) in comparison to $T_1$ and $T_2$, but no difference was detected between the first two sampling times (Figure 3).

Discussion

The mechanisms related to the secondary effects of anaesthesia in patients undergoing surgery are still not completely understood. Thus, we evaluated the extent of DNA strand breaks, alkali-labile sites and oxidative damage and frequency of apoptosis in lymphocytes and gene expression in blood cells from individuals under ISF anaesthesia.

The lack of genotoxicity observed in the present study was in agreement with those negative results found when ISF was tested in S.typhimurium and D.melanogaster assays (13,14). Similarly, no increase of sister chromatid exchanges has been reported in Chinese hamster ovary (CHO) and lung (CHL) cells (32,33) and also in lymphocytes from patients under minor orthopaedic surgery (34). These data are in accordance with the International Agency for Research on Cancer, which classifies volatile anaesthetics as not classifiable as to their carcinogenicity to humans (Group 3) (35). However, positive ISF

Table I. DNA single- and double-strand breaks, alkali-labile sites (DNA damage) and oxidised bases (oxidative DNA damage) evaluated by comet assay in lymphocytes from patients exposed to surgery and ISF among the time points evaluated

<table>
<thead>
<tr>
<th>Tail intensity (%)</th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>DNA damage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_1$</td>
<td>19.0 (12.7–24.6)</td>
<td>17.8 (11.1–28.1)</td>
<td>18.5 (16.7–21.3)</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>$T_2$</td>
<td>31.8 (21.7–38.6)</td>
<td>29.6 (21.0–39.9)</td>
<td>22.9 (21.3–29.9)</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>$T_3$</td>
<td>22.3 (13.0–27.4)</td>
<td>21.8 (15.5–26.9)</td>
<td>14.5 (9.4–21.1)</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

$T_1$: before anaesthesia; $T_2$: 2 h after the beginning of anaesthesia and $T_3$: on the first post-operative day. Data, obtained from 20 subjects, are expressed as median and quartiles.

$^a$Determined as Fpg-sensitive sites.

$^b$Determined as endo III-sensitive sites.
genotoxicity has been also described. This anaesthetic, at concentrations of 1 and 10 mM, was able to induce DNA damage in lymphocytes in vitro although the lesions have been completely repaired after 60 min (15). Increased DNA damage has been also detected in some organs of rats exposed to 1% ISF, with no evident association between genotoxicity and lipid and protein oxidation (17).

In patients classified as ASA I and II (patients with mild systemic disease and disturbance due to surgical condition) aged from 22 to 66 years, submitted to invasive abdominal surgeries under ISF (1 and 1.5%) anaesthesia, increased DNA damage has been observed in lymphocytes collected 60 and 120 min after the beginning of anaesthesia and also at the day after anaesthesia (2,18). Differently from our results, the positive findings might be related to the recruited patients and/or type of surgery. Literature shows that some confounding factors, such as associated diseases (ASA II) and old age, can increase the level of DNA damage and genomic instability (36–39). In our study, strict criteria for selecting patients were used. All patients enrolled were young adults classified as ASA I, with normal body mass index and with no other associated disease or systemic disturbances. Additionally, all the patients were no smokers or alcohol, drugs and antioxidant supplements consumers. Our data were only obtained from individuals submitted to elective non-invasive (tympanoplasty or septoplasty) surgery, and all the drugs used during anaesthesia and for the post-operative analgesia have not been reported as genotoxics or cytotoxics (40,41). It is known that open surgery, such as abdominal ones, is more traumatic and usually associated with increased inflammatory response (42). As previously suggested (15), ISF can be metabolised into reactive products or generate ROS, which might reach DNA, causing damage. However, it must be considered that only 0.2% of the anaesthetic is metabolised (7). Thus, for the first time, our findings showed that ISF anaesthesia did not induce oxidative DNA damage as recognised by Fpg and endo III enzymes in the comet assay and did not alter the extent of DNA damage induced by the in vitro treatment with H₂O₂.

It is well known that healthy individuals differ in their intrinsic capability to repair genetic damage, mainly because of DNA repair gene polymorphisms or by alterations in the gene expression pattern (43). On the other hand, it has been demonstrated that inflammatory processes can also inactivate some repair enzymes (44). We have previously observed that patients submitted to surgery under ISF anaesthesia showed increased pro-inflammatory cytokine interleukin-6 concentrations 120 min after the beginning of anaesthesia, with more pronounced increase on the first post-operative day (M. A. Mazoti, M. G. Braz, M. A. Golim, L. G. Braz, N. H. Dias, J. R. Braz, D. M. Salvadori, D. Fecchio, in preparation). Therefore, the expression of hOGG1, which removes 8-oxoguanine, a well-known mutagenic lesion (45), and XRCC1, which is involved in the repair of single-strand breaks and base damage (46,47), was down-regulated on the first post-operative day possibly due to the inflammatory status of the patient. Nevertheless, it was not possible to directly associate gene expression profile with the amount of DNA lesions because the first was investigated in whole blood cells and damage was measured only in lymphocytes. Thus, the down-regulation of XRCC1 and hOGG1 might not necessarily reflect a decreased DNA repair ability.

In our study, we also evaluated the frequency of cells in early and late apoptosis in patients exposed to ISF during minor surgery. Data showed no increase of apoptosis in either CD4⁺ or CD8⁺ non-cultured T cells, suggesting no important effect of this type of anaesthesia on the immune system. Contrarily, in major surgery, the inflammatory stress response and the post-operative immunosuppression after inhalation anaesthesia seem to be characterised by peripheral T-cell lymphopenia and leucocytosis (48,49). It has been demonstrated that ISF, only at high concentrations (up to 2.5%) and long-term in vitro treatment, is able to induce dose- and time-dependent apoptosis in lymphocytes (23). Additionally, ASA I and ASA II patients undergoing invasive surgeries (aortic femoral bypass, aneurysmectomy and cholecystectomy) under ISF and nitrous oxide (N₂O) anaesthesia exhibited higher frequencies of CD4⁺ and CD8⁺ apoptosis, when assessed by the 7-AAD 24 h (but not 96 h) after surgery (24). Similarly, cancer patients undergoing surgery with ISF and N₂O anaesthesia presented increased frequency of apoptosis in cultured lymphocytes obtained 2 h after the beginning of the surgery (22). However, it was not possible to determine whether the effect was caused by ISF or by N₂O. So, N₂O was never used in the patients in the present study in order to avoid possible DNA damage and because of its immunosuppressive activity (3,50).

### Table II. DNA damage (%tail intensity) in lymphocytes of patients under anaesthesia with ISF collected at three time points and in vitro exposed to hydrogen peroxide

<table>
<thead>
<tr>
<th></th>
<th>T₁</th>
<th>T₂</th>
<th>T₃</th>
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<tbody>
<tr>
<td>Tail Intensity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes⁺</td>
<td>2.6 (1.3–3.8)</td>
<td>2.6 (1.5–6.7)</td>
<td>2.4 (1.4–3.5)</td>
</tr>
<tr>
<td>H₂O₂⁺</td>
<td>10.3 (4.6–12.8)</td>
<td>6.9 (2.7–7.8)</td>
<td>5.5 (2.9–9.4)</td>
</tr>
</tbody>
</table>

T₁: before anaesthesia; T₂: 2 h after the beginning of anaesthesia and T₃: on the first post-operative day. Data obtained from 20 subjects, as expressed as median and quartiles.

### Table III. Percentage of viable, early and late apoptotic helper and cytotoxic T lymphocytes from patients undergoing anaesthesia with ISF

<table>
<thead>
<tr>
<th>T lymphocytes</th>
<th>CD4⁺</th>
<th>CD8⁺</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>T₁</td>
<td>T₂</td>
</tr>
<tr>
<td>Viable</td>
<td>91.9 (89.3–93.7)</td>
<td>91.7 (88.1–93.2)</td>
</tr>
<tr>
<td>Early apoptosis</td>
<td>7.0 (4.8–9.8)</td>
<td>7.4 (5.5–11.0)</td>
</tr>
<tr>
<td>Late apoptosis</td>
<td>1.0 (0.7–1.2)</td>
<td>0.9 (0.6–1.3)</td>
</tr>
</tbody>
</table>

T₁: before anaesthesia; T₂: 2 h after the beginning of anaesthesia and T₃: on the first post-operative day. Data obtained from 20 subjects, as expressed as median and quartiles; P > 0.05.
Fig. 1. Relative expression of hOGG1 DNA repair gene in blood cells of patients under general anaesthesia with ISF. T1: before anaesthesia; T2: 2 h after the beginning of anaesthesia and T3: on the first post-operative day. The horizontal bars indicate median RQ in each sampling time; dotted lines indicate the limits of RQ 0.5 and RQ 1.5. Data were obtained from 18 subjects. P < 0.05: (T1 = T3) > T2.

Fig. 2. Relative expression of XRCC1 DNA repair gene in blood cells of patients under general anaesthesia with ISF. T1: before anaesthesia; T2: 2 h after the beginning of anaesthesia and T3: on the first post-operative day. The horizontal bars indicate median RQ in each sampling time; dotted lines indicate the limits of RQ 0.5 and RQ 1.5. Data were obtained from 18 subjects. P < 0.05: T1 < T2.

Fig. 3. Relative expression of anti-apoptotic BCL2 gene in blood cells of patients under general anaesthesia with ISF. T1: before anaesthesia; T2: 2 h after the beginning of anaesthesia and T3: on the first post-operative day. The horizontal bars indicate median RQ in each sampling time; dotted lines indicate the limits of RQ 0.5 and RQ 1.5. Data were obtained from 18 subjects. P < 0.05: (T1 = T3) > T2.

Down-regulation of pro-apoptotic p53 and anti-apoptotic protein Bcl-2 has been reported 1 day after the surgery in patients under anaesthesia with ISF (24). Similarly, we also detected low expression of this anti-apoptotic gene transcript on the first post-operative day. Perhaps, BCL2 is repressed 1 day after surgery to allow damaged cells to undergo apoptosis. Aravindan et al. (51) have found reduced expression of death receptors, BCL2, TP53 and ATM genes in renal cells after 2% ISF, showing an inhibition of apoptosis in rats. These authors have suggested that ISF has anti-apoptotic activity mediated via both extrinsic and intrinsic signalling pathways. It is believed that short period under anaesthesia with ISF might provide cytoprotection via preconditioning, whereas prolonged exposures produce direct cytotoxicity (52). It has been also demonstrated that exposure to ISF enhances Bcl-2 protein in rodent cardiomyocytes in response to hypoxia, while H2O2 decreases Bcl-2 production (26).

In conclusion, the current study supports the observation that general anaesthesia maintained with ISF does not induce DNA strand breaks, alkali-labile sites or oxidative damage, neither increases the percentage of apoptotic CD4+ and CD8+ in lymphocytes or changes genes expression in blood cells in ASA I patients undergoing elective non-invasive surgery. Nevertheless, DNA repair and apoptosis-related genes were down-regulated on the first post-operative day.

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Conflict of interest statement: None declared.

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