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Occupational exposure to anesthetics leads to genomic instability, cytotoxicity and proliferative changes

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ABSTRACT

Data on the genotoxic and mutagenic effects of occupational exposure to the most frequently used volatile anesthetics are limited and controversial. The current study is the first to evaluate genomic instability, cell death and proliferative index in exfoliated buccal cells (EBC) from anesthesiologists. We also evaluated DNA damage and determined the concentrations of the anesthetic gases most commonly used in operating rooms. This study was conducted on physicians who were allocated into two groups: the exposed group, which consisted of anesthesiologists who had been exposed to waste anesthetic gases (isoflurane, sevoflurane, desflurane and nitrous oxide – N_2O) for at least two years; and the control group, which consisted of non-exposed physicians matched for age, sex and lifestyle with the exposed group. Venous blood and EBC samples were collected from all participants. Basal DNA damage was evaluated in lymphocytes by the comet assay, whereas the buccal micronucleus (MN) cytome (BMCyt) assay was applied to evaluate genotoxic and cytotoxic effects. The concentrations of N_2O and anesthetics were measured via a portable infrared spectrophotometer. The average concentration of waste gases was greater than 5 parts per million (ppm) for all of the halogenated anesthetics and was more than 170 ppm for N_2O, expressed as a time-weighted average. There was no significant difference between the groups in relation to lymphocyte DNA damage. The exposed group had higher frequencies of MN, karyorrhexis and pyknosis, and a lower frequency of basal cells compared with the control group. In conclusion, exposure to modern waste anesthetic gases did not induce systemic DNA damage, but it did result in genomic instability, cytotoxicity and proliferative changes, which were detected in the EBC of anesthesiologists. Thus, these professionals can be considered at risk for developing genetic alterations resulting from occupational exposure to these gases, suggesting the need to minimize this exposure.

1. Introduction

Human monitoring has been used as a useful tool to identify and quantify the risks related to harmful effects on health. Several studies have reported detrimental effects in professionals who work in hospitals due to their occupational exposure to potentially dangerous conditions, such as ionizing radiation, toxic reagents, anticancer drugs and anesthetic gases utilized in the operating rooms (ORs) [1–4].

Prominent among the numerous known occupational hazards related to the practice of anesthesiology is the toxicity of volatile anesthetics, which have attracted special attention because of their wide clinical use and their potential to cause genetic damage in exposed professionals. However, previous studies on occupational exposure to anesthetics and systemic DNA damage detected based on different endpoints (comet assay, micronucleus – MN, sister chromatid exchanges – SCE or chromosome aberrations – CA) have provided inconsistent findings; there are some reports demonstrating a positive relationship between exposure to anesthetics and the incidence of genome damage [5–7], while others did not find this association [8–10]. Hence, determining the specific undesirable health effects caused by anesthetic gases in humans is of special concern.

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A validated method for detecting a biomarker of genomic instability is the human buccal MN cytome (BMCyt) assay [11], which is the preferred choice for the biomonitoring of genetic damage in humans. Some advantages of this method include minimally invasive cell collection and ease of storage and slide preparation [12]. Additionally, the BMCyt assay is an approach that can be used for measuring DNA damage, cell proliferation, cell differentiation and cell death in exfoliated buccal cells [13]. However, only a single study has evaluated buccal MN in professionals exposed to anesthetic gases [14]. In addition, no study has yet evaluated the cell types and other nuclear abnormalities that are scored in the BMCyt in response to the occupational exposure to waste anesthetic gases.

The alkaline comet assay is also an established and valuable tool for detecting DNA lesions in the human monitoring field. This assay, also known as the single-cell gel electrophoresis (SCGE) assay, consists of immersing the eukaryotic cells in agarose gel to lyse the cell membrane with detergents and alkaline salts, and detecting damaged DNA based on the differential migration of the nuclear material when subjected to electrophoresis [15]. Unfortunately, most of the previous studies on DNA damage and occupational exposure to anesthetics did not determine the ambient-air gas concentrations in the ORs [5,16,17].

To minimize risks, health authorities recommend limits on exposure to waste anesthetic gases. The National Institute of Occupational Safety and Health from the U.S.A. (NIOSH) [18] suggests an exposure ceiling of 2 parts per million (ppm) for volatile anesthetics and 25 ppm for nitrous oxide (N_2O), expressed as a time-weighted average (TWA) over the duration of exposure. It should be emphasized that most developing countries do not have recommended limits for waste-anesthetic-gas exposure.

To the best of our knowledge, this is the first study to assess the cell types and nuclear alterations in the exfoliated buccal cells and systemic DNA damage in anesthesiologists and to measure the levels of the most common anesthetics currently used in the ORs in a tertiary teaching hospital.

2. Materials and methods

The study was performed at Sao Paulo State University Hospital (UNESP, Botucatu, Brazil) after receiving approval from the Human Research Ethics Committee of the Botucatu Medical School-UNESP (n. 417.865). The study was registered in the Brazilian Clinical Trials Registry (RBR-4vrz2m). All of the physicians who participated in this study worked at the same hospital and provided written informed consent.

2.1. Study design

Sixty subjects were enrolled in this study and were allocated into two groups. The first group consisted of 30 anesthesiologists who had been exposed to the following waste anesthetic gases for at least two years: isoflurane, sevoflurane, desflurane and N_2O. The control group was comprised of 30 internal medicine physicians without occupational exposure to the waste anesthetic gases, who were matched for age, sex and lifestyle with the exposed group. All subjects answered a detailed questionnaire that inquired about their demographic data (age, sex, body mass index – BMI, etc.), medical history (vaccinations, medications, diseases, etc.), lifestyle (diet, physical exercise, alcohol and tobacco consumption, etc.), and occupational exposure, as well as other topics. Pregnant women; individuals with malignant, infectious or inflammatory diseases; heavy drinkers or smokers; or those subjects taking medications, vitamins and/or antioxidant supplements were excluded from the study to avoid possible confounding effects.

2.2. Collection of biological samples

To avoid any possible bias, all samples were collected not only concomitantly in the control and exposed groups before the physicians left for a vacation, but also during the same period in the morning after the participants had fasted for 8 h. In addition, the participants were instructed to suspend the use of mouthwash (antiseptic solutions) for at least three days before biological sample collection.

Venous blood samples were collected in tubes containing EDTA as an anticoagulant. Prior to collection of buccal cells, all individuals were asked to perform three vigorous buccal rinses using warm water to eliminate any waste material. Buccal cells from both sides of the oral cavity (cheek) and the lower lip were obtained by using a cytobrush that was placed in a 15-mL tube containing a cold saline solution (PBS). All samples were coded and immediately processed under yellow light to prevent the possibility of additional damage.

After blood collection, peripheral blood lymphocytes (PBLs) were isolated by means of the Ficoll-Paque® gradient method, following the previously described protocol [19]. After isolation, lymphocytes were gradually frozen in Roswell Park Memorial Institute (RPMI) medium, fetal bovine serum and dimethyl sulfoxide (DMSO) and kept at -80°C until assayed [20].

To avoid possible bias, the samples from the control and exposed subjects were always mixed in each batch of the assays, and therefore the matched samples were always subjected to the same conditions in all tests steps, and at the same time.

2.3. Comet assay (single-cell gel electrophoresis assay)

The alkaline comet assay was performed to assess baseline DNA damage in both groups according to the techniques described by Singh et al. [21] and Tice et al. [22], with slight modifications [23].

The lymphocytes were thawed gradually and a volume of 10 μL was added to 120 μL of 0.5% low-melting-point (LMP) agarose in PBS at 37°C. These mixtures were pipetted onto two microscopy slides, which had been previously precoated with a layer of 1.5% normal agarose, covered with coverslips and left for 8 min at 4°C. After agarose solidification, the coverslips were discarded and the slides were placed into a cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris at pH 10 with 1% Triton X-100 and 10% DMSO) for 2 h. After lysis, the slides were washed in PBS and placed in an acrylic horizontal electrophoresis tank that contained freshly prepared alkaline buffer (1 mM EDTA and 300 mM NaOH at pH > 13) for 20 min to promote DNA unwinding. The electrophoresis (PowerPac™ HC Power Supply, Bio-Rad Laboratories, Brazil) was performed at 0.8 V/cm and 300 mA for 20 min. Lymphocytes isolated from healthy volunteers were used to prepare the negative and positive control slides in each run. After electrophoresis, the slides were immersed for 15 min in neutralization solution (0.4 M Tris at pH 7.5), fixed with absolute ethanol and stored at 4°C until analysis.

Two slides for each individual were stained with 80 μL SYBR Gold and a total of 100 randomly captured nucleoids per individual (50 from each slide) were blindly analyzed at 400 x magnification using a fluorescent microscope connected to an image analysis system (Comet Assay IV, Perceptive Instruments, UK). Tail moment and tail intensity were used to estimate DNA damage. As tail intensity gave similar results, only tail moment (a.u.) values were presented.

2.4. Buccal micronucleus cytome assay (BMCyt assay)

The procedures followed the protocol described by Tolbert et al. [24], with slight modifications. Immediately after buccal cells were collected, the falcon tube was vortexed and centrifuged for 5 min at 1000 rpm. The supernatant was removed and the pellet was