Mutat Res Gen Tox En 832–833 (2018) 61–64

Contents lists available at ScienceDirect

Mutat Res Gen Tox En

journal homepage: www.elsevier.com/locate/gentox

Short communication

Detrimental effects detected in exfoliated buccal cells from anesthesiology medical residents occupationally exposed to inhalation anesthetics: An observational study

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ARTICLE INFO

Keywords:
Occupational exposure
Anesthetic gases
Buccal mucosa
Genome instability

ABSTRACT

Operating room professionals are scarcely aware of their individual occupational exposure to waste anesthetic gases (WAGs). Medical residents spend several hours per day in operating rooms and consequently experience occupational exposure to WAGs. Considering that no studies have yet evaluated the potential toxicity in medical residents exposed to WAGs using the buccal micronucleus cytome (BMCyt) assay, this pioneering study aimed to compare the BMCyt assay markers, including DNA damage, cell proliferation, and cell death in the exfoliated buccal cells of surgery and anesthesiology residents occupationally exposed to WAGs. The study enrolled a total of 60 physicians, including internal medicine residents (unexposed group), and residents from surgery and anesthesiology programs who were occupationally exposed to sevoflurane, isoflurane and nitrous oxide. WAGs were measured, and the mean values were higher than the international recommendation. The anesthesiology residents (high exposure) showed statistically significant lower frequencies of basal cells, and statistically significant higher frequencies of micronuclei, karyorrhexis, pyknosis, and differentiated cells than did the unexposed group; karyolysis frequencies were significantly higher in anesthesiology residents than were those in the unexposed group or in surgical residents (low exposure). The findings suggest a genetic risk for young professionals exposed to WAGs at the beginning of their careers. Thus, exposure to high WAGs concentrations leads to impairment of the buccal cell proliferative potential, genomic instability and cell death, especially in anesthesiology residents, demonstrating an early impact on their health.

1. Introduction

The most important cause of waste anesthetic gases (WAGs) contamination in a surgical environment is the lack of a proper scavenging system in the operating rooms [1]. However, even if professionals are working in scavenged operating rooms, they are still exposed to trace concentrations of inhalation anesthetics, particularly during pediatric anesthesia [2]. Thus, several types of professionals are exposed daily to WAGs, and this exposure can have harmful effects on workers' health [3,4]. Although inhalation anesthetics have been commonly used for some decades in clinical practice, operating room professionals are scarcely aware of their individual occupational exposure to WAGs.

According to the National Institute of Occupational Safety and Health (NIOSH) [5,6] the recommended exposure limit to minimize risk is 25 ppm as the time-weighted average (TWA) for the anesthetic gas nitrous oxide and 2 ppm for a one-hour ceiling for old halogenated anesthetics, such as halothane and enflurane. However, the exposure limit for most modern inhalation anesthetics (halogenated), such as isoflurane, sevoflurane and desflurane, remains an open question.

The potential of WAGs to induce systemic genetic damage has already been described [7,8]. However, these previous studies were performed in professionals exposed to an unmeasured mixture of anesthetic concentrations, including halothane, which is currently not in use.

Among several techniques for assessing DNA damage, the human buccal micronucleus (MN) cytome (BMCyt) assay has a low cost, is

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https://doi.org/10.1016/j.mrgentox.2018.07.001
Received 20 February 2018; Received in revised form 20 June 2018; Accepted 2 July 2018
Available online 03 July 2018
1383-5718/

simple to perform, does not require cell culture and is minimally invasive [9]. This assay is a cytogenetic method for measuring genetic damage, cell proliferation and cell death in exfoliated buccal cells and has been widely used for biomonitoring of inhalation exposure [10]. In addition, buccal cells serve as one of the first barriers in direct contact with inhalation substances, such as WAGs. However, only two studies have applied this assay to evaluate the toxic effects of chronic exposure to WAGs [7,11].

Anesthesiology and surgery residents spend several hours per day in operating rooms and consequently have occupational exposure to WAGs. Indeed, a few studies have reported genetic lesions, changes in redox status and increased pro-inflammatory cytokines in these young professionals exposed to WAGs [12,13]. However, no studies have yet evaluated cell toxicity in medical residents exposed to WAGs using the BMCyt assay.

Considering the novelty and importance of this subject, the current pilot study aimed to compare markers of DNA damage, cell proliferation, and cell death in exfoliated buccal cells from young physicians occupationally exposed to WAGs at the end of their anesthesia or surgery residency programs.

2. Material and methods

2.1. Study design and occupational exposure

After receiving approval from the Human Research Ethics Committee (38250114.3.0000.5411), and after obtaining written informed consent from all the participants, this observational study recruited medical residents from a Brazilian tertiary hospital as follows: 30 residents from internal medicine (unexposed group) and 30 residents from surgery (n = 16) and anesthesiology (n = 14) programs (exposed to the WAGs sevoflurane, isoflurane and nitrous oxide). A questionnaire was administered to all the individuals to obtain demographic data and lifestyle and medical history. The exposed group was age- and sex-matched with the unexposed group. The biological sampling was performed from 2013 to 2014. Current smokers, heavy drinkers [those who consumed more than one liter of light beverage or two cups of hard liquor per day for at least six years; [14], individuals who had chronic infectious or inflammatory diseases, and those who used medications, vitamins and/or antioxidant supplements, and those who had recently been exposed to radiation (within a month) were excluded from the study to avoid possible bias.

All the medical residents from the exposed group worked in all the 13 operating rooms in the hospital, of which half had a partial scavenging system and the other half had no active scavenging system, as previously described [15]. All the WAGs were measured (ppm) at real time in the medical resident's breathing zone during surgeries under inhalation anesthesia in all the operating rooms. The measurements were performed using a portable infrared analyzer (InfraRan 4-gas anesthetic specific vapor, Wilks Enterprise, East Norwalk, CT), which was calibrated, as recommended by the manufacturer, prior to the measurements. TWA values (for nitrous oxide) and averages (for the halogenated anesthetics) were calculated and shown in ppm.

2.2. Biological samples and the BMCyt assay

The BMCyt assay was performed according to a previously described protocol [16] with slight modifications. Briefly, fresh exfoliated buccal cells were collected from both cheeks of the medical residents using a wooden spatula that was subsequently placed in a coded falcon tube containing cold saline solution. The tube was vortexed for detachment of the cells and then centrifuged. The supernatant was removed, and the cells were fixed with methanol-acetic acid solution. The pellet was suspended and used to prepare coded slides in duplicate per individual. Then, the slides were stained using the Feulgen-fast green method.

All the cell types and nuclear abnormalities from the buccal mucosa samples were determined as recommended [17] by blind analysis using an Olympus BX43 microscope at x 1000 magnification. One experienced person scored the slides and cells were scored using bright field, and cells containing MN were confirmed as being positive by examining the cells under fluorescence. The frequencies of the various cell types were scored per 1000 cells, while the frequencies of MN and nuclear buds were scored per 2000 differentiated cells.

2.3. Statistical analysis

The sample size was calculated based on a pilot study (MN marker) to provide 80% statistical power (type I error - α of 0.05 and a type II error - β of 0.20) to detect a mean group difference of 0.4 and standard deviation of 0.55. The demographic data (age and body mass index) were analyzed using an analysis of variance, and sex was analyzed using a chi-square test. A generalized linear model (Poisson regression or negative binomial) was used for the analyzed BMCyt parameters. The results are expressed as the means and standard deviations. Significance was set at $p < 0.05$.

3. Results

Because the groups were matched, there were no significant differences regarding demographic data (Table 1). All the physicians were young adults (25–33 years of age) of both sexes. The anesthesiology residents spent approximately 60 h/week (high hours exposure), and surgery residents spent 18 h/week (low hours exposure) in the operating rooms. The exposure assessment demonstrated that trace concentrations were 155 ± 138 ppm for nitrous oxide, 9.8 ± 9.0 ppm for sevoflurane and 5.1 ± 4.2 ppm for isoflurane.

Table 2 shows the detrimental effects observed in the exfoliated buccal cells (BMCyt assay) from the evaluated physicians. The exposed anesthesiology residents showed lower basal cell frequencies and higher differentiated cells frequencies than the control group. MN frequency was increased in the anesthesiology residents compared with that in the unexposed residents ($p = 0.038$). Karyorrhexis (2.5-fold) and pyknosis (3.1-fold) were significantly increased in the anesthesiology residents compared with those in the control group. Despite the higher frequencies were observed in the residents from the anesthesiology field and to a lesser degree from the surgery field, there were no significant differences among the groups in nuclear buds, binucleated cells or condensed chromatin ($p > 0.05$). Karyolysis was found to be significantly higher in anesthesiology medical residents than that in unexposed residents (4.9- fold) and surgery residents (2-fold).

4. Discussion

This pilot study showed for the first time that medical residents occupationally exposed to WAGs, especially the anesthesiology residents, showed alterations in buccal cell regenerative potential, demonstrated by the reduced frequency of basal cells and increased MN formation and cell death biomarkers, which are related to genetic

Table 1
Demographic data.

Parameters	Unexposed (n = 30)	Exposed group	
		Anesthesiology (n = 14)	Surgery (n = 16)
Age (years)	28.8 ± 1.9	28.9 ± 1.8	28.2 ± 1.4
Sex (male)	18	9	9
Body mass index (kg/m^2)	24.7 ± 3.9	24.3 ± 3.2	24.9 ± 4.4
Duration of exposure (years)	–	2.9 ± 0.4	3.2 ± 0.5

Data expressed as means ± standard deviation or number. $p > 0.05$.