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Genotoxicity of occupational exposure of anesthesiologists to sevoflurane

— The relationship between DNA damage and the work environment —

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ABSTRACT

Background: Inhalation anesthetics are often used for general anesthesia. However, several studies have reported that occupational exposure to inhalation anesthetics induces DNA damage and that sevoflurane anesthesia causes reversible genotoxic effects in patients. Our aim was to investigate the genotoxic effects in anesthesiologists of occupational exposure to sevoflurane, and to reveal the relevance of DNA damage and the work environment of anesthesiologists.

Methods: Alkaline comet assay of the peripheral blood lymphocytes of 53 anesthesiologists (exposed group) and 37 office workers (control group) was performed, following which 100 cells were classified into 5 classes: 0: no migration, to 4: very high migration, the distance of migration being dependent on the degree of DNA damage. The total comet score (TCS) was calculated as n1+2×n2+3×n3+4×n4. Anesthesiologists were asked about their work environment.

Results and Conclusions: TCSs were no different between the exposed and control groups (7.16±7.88 vs. 7.63±6.73, p=0.82) (95%CI 3.91-10.41 vs. 4.97-10.29). There was no significant association between TCS and the frequency of usage of sevoflurane. In conclusion, our study did not reveal DNA damage with chronic occupational exposure to sevoflurane.

Key words : occupational exposure, sevoflurane, comet assay, DNA damage

Introduction

Inhalation anesthetics are often used for general anesthesia. However, several studies have reported that occupational exposure to inhalation anesthetics induces DNA damage and that sevoflurane anesthesia causes reversible genotoxic effects in patients.

In the 1960s, occupational exposure to wasted anesthetics was reported to cause adverse pregnancy outcomes in health-care personnel,[1] with many studies reporting a greater incidence of spontaneous abortions and birth defects in anesthesiologists.[2)−9)] Thereafter, public health authorities recommended threshold values for wasted anesthetics, to minimize the possible health risks.[10] The subsequent widespread use of anesthetic gas scavenging systems resulted in a considerable decrease in the exposure of operating staff to these gases. However, it is still possible to be exposed to volatile anesthetics, such as during bag-mask ventilation.

Rozgaj et al.[11] reported that the tail length of lymphocyte migration in technicians who worked in operation rooms was significantly increased compared

with controls. They also found that micronucleus frequency was significantly increased in the exposed group. There was also a significant increase in sister chromatid exchange (SCE) values in anesthesiologists compared with non-exposed physicians.[12] However, they reported that the increase in SCE values was reversible. Other reports have indicated no significant differences in the results of comet assay between exposed and non-exposed subjects.[13]

Furthermore, reversible genotoxic effects in patients exposed to sevoflurane anesthesia have been reported.[14)–15)] DNA single strand breaks were detected in peripheral blood lymphocytes of malignant breast cancer diagnosed patients under sevoflurane anesthesia. Total comet score which expressed the magnitude of DNA damage increased significantly at 120 min of anesthesia and recovered 5 days after the operation.[15] Karabıyık et al.[16] investigated the comet response of the patients who underwent elective lower abdominal surgery under sevoflurane anesthesia. There were significant increases in the mean comet response at 60 or 120 min of anesthesia and on the first day after anesthesia. Removal of the DNA damage was observed after the third day of anesthesia and the repaired was completed within 5 days. On the other hand, genetic damage of Swiss albino mice cells was investigated by the comet assay in their reports.[17] Mice were exposed to sevoflurane. Significant DNA damage immediately after exposure was observed in leukocytes. There were no revealed signs of repair until 24h after the last exposure.

However, the relationship between DNA damage and the frequency of use of volatile anesthetics and the occupational exposure dose of anesthetics has not been reported. In addition, volatile anesthetic concentrations that operating room personnel are exposed to are not clear enough. The aim of our study was to investigate the genotoxic effects in anesthesiologists of occupational exposure to sevoflurane, and to reveal the relevance of DNA damage and the work environment of anesthesiologists. Additionally, we measured sevoflurane concentrations in operation rooms.

Methods

After obtaining written informed consent from the study participants and the approval of the Institutional Review Board of Yamagata University Faculty of Medicine, 90 volunteers, 53 anesthesiologists (exposed group) and 37 office workers (non-exposed group), were included in this study.

The exposed group included anesthesiologists who worked in operating rooms of hospitals in Yamagata Prefecture. The non-exposed group, serving as a control, included office workers who worked in offices in hospitals. All study subjects completed a questionnaire that asked about their previous history, smoking habits, exercise habits, hours of sleep, overtime work, vaccinations and exposure to pollutants, as well as diagnostic and therapeutic radiography during the previous month. In addition, anesthesiologists were asked about the frequency of usage of each anesthetic, each airway management tool, and the techniques used to avoid leakage of volatile anesthetics, such as when filling the vaporizer, use of endotracheal tube cuff pressure monitoring, etc.

Cell preparation

Five-millilitre samples of venous blood were collected in heparinized tubes from all volunteers and processed within 3 h. All the samples were coded and blindly analyzed. For this, 5 mL of heparinized blood mixed with RPMI 1640 medium (Sigma-Aldrich Co., Ltd., Tokyo, Japan) was carefully layered over 3 mL of Lymphoprep™ (COSMO Bio Co., Ltd., Tokyo, Japan) and centrifuged for 20 min at least 2000 *g*. The interface band containing lymphocytes was washed with RPMI and collected by 10 min centrifugation at 1500 *g*. Cells were checked for viability by the trypan blue exclusion test. The specimen was included in the study if the viability rate of lymphocytes was greater than 90%.

Slide preparation

Alkaline comet assay was performed according to

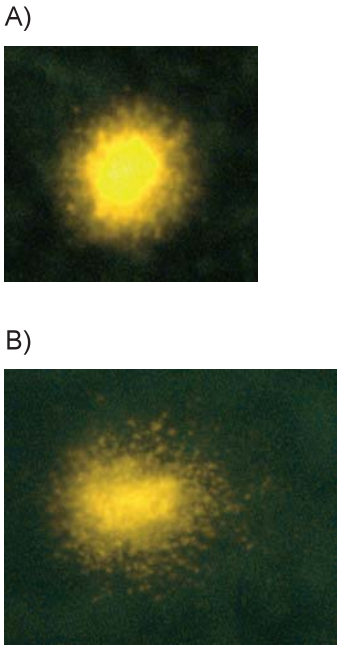

Figure 1.
A) An undamaged nucleus of peripheral blood lymphocyte (no migration)
B) A damaged nucleus of peripheral blood lymphocyte (high migration)

the method of Singh et al.[18] with the following modification. One hundred and fifty microlitres of lymphocyte suspension was mixed with 150 uL of 2% low-melting point agarose and rapidly pipetted onto pre-cleaned microscope slides previously pre-coated with 200 uL of 1% normal-melting point agarose, and immediately covered with a cover slip. After the agarose gel had solidified, the cover slip was removed and the slides were immersed in lysing solution consisting of 2.5 M NaCl, 100mM EDTA, 10mM Tris and 1% sodium sarcosinate, with an adjusted pH of 10 with 1% TritonX-100 and 10% DMSO, for at least 1 h.

Electrophoresis

The slides were placed in a freshly prepared alkaline buffer (300 mM NaOH, pH>13) and left for 20 min to allow unwinding of DNA and expression of alkali-labile damage. Electrophoresis was carried out in the same solution for 20 min at 25V (300-400 mA) and 0 ℃. The slides were then neutralized with Tris buffer, (pH 7.5) and stained with 200 uL of ethidium bromide (2 uL ml-1). The slides were analyzed using a fluorescence microscope (magnification ×400) with an excitation filter of 510-560 nm.

Images of 100 randomly selected lymphocytes (50 cells from each of two derived slides) were analyzed for each sample. The parameter assessed was tail length, which is the distance of migration that is dependent on the degree of DNA damage (Figure 1). Images were classified into 5 classes (0-4), where 0 indicates no migration and 4 indicates very high migration, with the distance of migration being dependent on the degree of DNA damage.

The number of cells in each migration class was counted and the total comet score (TCS) was calculated as follows: TCS=n1+2×n2+3×n3+ 4×n4.

Statistical analyses

Characteristics and TCSs between groups and the association between TCS and the frequency of inhalational anesthetic gas usage in the exposed group were compared using Chi-squared and t-tests. The association between TCS and the frequency of inhalational anesthetic usage in the exposed group was analyzed using Pearson's product-moment correlation coefficient. P-values less than 0.05 were considered significant. All analyses were performed using SAS 9 (SAS, Cary, NC, USA).

We estimated the sample size based on a difference among groups in the TCS by 26 with a standard deviation (SD) of 5. On the basis of these assumptions, we calculated that 3 patients per group were required to achieve 80% power at 5% α error. Although the image of tail length were classified into 3 classes (0-2) in most of previous study, they were classified into 5 classes to detect small differences in this study. S.Sardas et al.[19] reported that the difference of TCS between anesthetic staffs and controls is 13, and SD is 5.0. The expected TCS is 26 which is double of 13.

Results

Subject flow chart are shown in Table 1. 90 subjects were enrolled this study, however smoking and recent

Table 1. Subject flow chart.

	Exposed	Control
enrollment	53	37
excluded (smoker/recentvaccination)	28	10
analysed	25	27

Table 2. Characteristics and total comet scores (TCS) in non-smokers and subjects without recent vaccination. SD, Standard Deviation ; BMI, Body Mass Index ; UV, ultraviolet ; TCS, Total Comet Score ; CI, Confidence interval

	Exposed (n=25)	Control (n=27)	P-value
Age (years)	39.0 ± 10.1	40.9 ± 11.0	
BMI (kg/m^2)	21.9 ± 2.1	22.1 ± 2.7	
Duration of exposure (months)	148.3 ± 118.4	0	
UV exposure (h/month)	3.4 ± 6.7	9.1 ± 18.7	
Hours of sleep (h/day)	6.0 ± 0.7	6.4 ± 0.8	
Male n (%)	13 (52.0)	11 (40.7)	
Exercise habit n (%)	6 (24.0)	5 (18.5)	
TCS (95%CI)	7.16 ± 7.88 (3.91-10.41)	7.63 ± 6.73 (4.97-10.29)	0.82

Table 3. Association between TCS and the frequency of usage of different devices and practices in the exposed group.

	Frequency (days/month)	Correlation coefficients	95%CI
Sevoflurane	8.4 ± 7.0	0.01	-0.18 - 0.35
Double lumen tube	3.0 ± 2.5	-0.06	-0.62 - 0.79
Laryngeal mask	3.3 ± 2.8	-0.15	-0.83 - 0.70
Uncuffed endotracheal tube	1.7 ± 2.1	-0.22	-1.17 - 0.62

Table 4. Association between TCS and the techniques of anesthesia. CI, Confidence interval

	n (%)	TCS Mean ± SD (95%CI)	P-value
Fill the vaporiser			0.19
Myself	13 (52.0)	5.15 ± 7.73 (0.48 - 9.83)	
Done by others	12 (48.0)	9.33 ± 7.76 (4.40 - 14.27)	
Fresh gas flow/concentration during intubation			0.86
Maintained	18 (72.0)	7.33 ± 8.16 (3.28 - 11.39)	
Reduced	7 (18.0)	6.71 ± 7.70 (-0.40 - 13.83)	
Endotracheal tube cuff pressure monitoring			0.006
Used	15 (60.0)	3.80 ± 5.72 (0.63 - 6.97)	
Never used	10 (40.0)	12.20 ± 8.22 (6.32 - 18.08)	

vaccination are considered to be factors that affect genotoxicity. Therefore, after excluding smokers and/or subjects with a recent history of vaccination, analyses were performed (case n = 25, control n = 27). The characteristics and TCS of the exposed and control groups are shown in Table 2. All the subjects periodically received a physical examination, and none of them were exposed to therapeutic radiation, chemotherapy or carcinogens, such as asbestos and organic solvents. There were no significant differences in TCS between the exposed and control groups (7.16±7.88 vs. 7.63±6.73, p=0.82) (95%CI 3.91-10.41 vs. 4.97-10.29). We also found no significant association between TCS and the frequency of sevoflurane usage in the exposed group (Table 3).

Thereafter, we investigated the association between

TCS and common practices during anesthesia. TCS in anesthesiologists who used endotracheal tube cuff pressure monitoring was significantly decreased compared to TCS in anesthesiologists who never used it (3.80±5.72 vs.12.20±8.22, p=0.006) (95%CI 0.63-6.97 vs. 6.32-18.08) (Table 4). Multiple regression analysis was performed with a factor of Table 3 and Table 4. However, it showed no significant influence for the use of cuff pressure monitoring devices on TCS (p=0.14, 95%CI -0.66-7.44). Since Total intravenous anesthesia was considered a confounding factor, it was excluded from analyses.

Discussion

In this study, we found that there was no significant difference in TCS between anesthesiologists and control group subjects. Further, we did not detect an association between TCS and the frequency of sevoflurane usage in the exposed group. However, TCS was significantly increased in anesthesiologists who never used endotracheal tube cuff pressure monitoring compared to TCS in those who did. However, multiple regression analysis showed no significant influence of the use of cuff pressure monitoring devices on TCS.

Previous studies found that the lymphocyte migration tail length of technicians in operation theatres was significantly high.[11] However, our study showed that there was no significant increase in genotoxic effects in exposed anesthesiologists. This difference could be because the exposure dose of anesthesiologists has considerably decreased recently, because inhalation anesthetics are not used as much as before and most operating rooms in Japan have air conditioning and scavenging systems. In fact, the sevoflurane concentration was less than the recommended exposure limit during most of the study period. Although the sevoflurane concentration was high at the time of induction, the concentration reduced speedily after induction of anesthesia. We believe that this short-term exposure to a high sevoflurane concentration does not produce genotoxic effects.

It has revealed that occupational exposure to sevoflurane and isoflurane is not associated with DNA damage, while exposure to nitrous oxide causes DNA damage.[20] Other studies have also demonstrated the genotoxicity of nitrous oxide. In recent years, remifentanil has become extremely common for perioperative analgesia. We rarely use nitrous oxide in our hospital, and the only volatile anesthetics used in most institutions in this study was sevoflurane. This enabled evaluation of the genotoxic effects of sevoflurane.

Some previous studies also reported DNA damage in patients undergoing surgery under volatile anesthesia. Total comet scores increased significantly in peripheral blood lymphocytes of patients undergoing breast cancer surgery under sevoflurane anesthesia (0.6-0.9 MAC, mean of the duration:130.67 min). However, DNA damage was detected at 120 min of anesthesia and recovered 5 days after the operation.[15] DNA damage in peripheral blood lymphocytes was also detected in patients undergoing elective lower abdominal surgery under sevoflurane anesthesia (0.6-0.9MAC, duration of anesthesia: 141.5±22.1min), repair of the damaged cells being complete by the fifth postoperative day.[16] Brozovic et al.[17] demonstrated transient DNA damage after sevoflurane anesthesia in peripheral blood lymphocytes of Swiss albino mice (2.4 vol % for 2h daily, for 3 days). On the other hand, DNA damage following sevoflurane or isoflurane anesthesia was not observed in some studies.[21), 22)] 40 children undergoing minor surgical procedures was maintained with sevoflurane at 1.4±0.77MAC.[21] Patients who underwent minimally invasive surgery was maintained with sevoflurane at 1-1.2MAC.[22]

The exposure dose of anesthesiologists would be much lower than that of patients who receive inhalation anesthetics because the amount of inhalation anesthetics is less than in previous years and most operating rooms in Japan have air conditioning and scavenging systems. Although anesthesiologists are exposed to high doses of sevoflurane during intubation, any DNA damage that occurs recovers quickly because the effects are reversible. This study suggests that anesthesiologists' exposure to sevoflurane with normal usage is sufficiently low and results in no significant increase in TCSs. Our results support the

safety of occupational exposure of anesthesiologists to sevoflurane.

In this study, control subjects were not matched to exposed subjects. As seen in the questionnaire, the genotoxic factors that differed significantly between exposed and control groups were smoking habit and recent vaccination. Therefore, after smokers and subjects with recent vaccination were excluded, we did not detect a significant differences between the two groups.

During analyses, we also divided all subjects into smokers and non-smokers, and into subjects with and without recent vaccination (results not shown). Although we compared TCS between each set of groups, we did not find significant effects of smoking and vaccination. Further, the Brinkman index, which is an indicator of smoking history, was <100 in most subjects. Thus, the effects of smoking could be eliminated.

We evaluated occupational genotoxicity by using the comet assay, which is a simple genotoxic test. This method has proven accurate in detecting mutagens and with a sensitivity equal to that of the micronucleus test.[23] The comet assay is commonly used to measure DNA damage as a marker of exposure to genotoxic agents or to investigate genoprotective effects in human.[24]

We investigated whether there were any factors related to the genotoxic effects of inhaled anesthetics, such as the frequency of usage, use with laryngeal masks, and anesthesiologists' usual practices. This is the first study to reveal the association between genotoxicity and anesthetic practices as chronic toxicity with circumstance pollution. Although we found no relationship between TCS and the frequency of sevoflurane usage, univariate analysis showed a significant increase in TCS in anesthesiologists who never used endotracheal tube cuff pressure monitoring. However, we could not detect any association between techniques likely to lead to leakage of volatile agents, such as use of uncuffed endotracheal tubes, laryngeal masks and double lumen tubes, and TCS. This may imply that it is important to reduce exposure to inhalation anesthetics by using endotracheal tube cuff monitoring because there are many opportunities to use endotracheal tube with cuff.

There are some limitations to this study. First, our study was not controlled for all genotoxic factors. There were no significant differences between the two groups after the exclusion of smokers and those who had been recently vaccinated. Further, we excluded subjects who had been exposed to formaldehyde and asbestos, although the effects of automobile exhaust fumes and powder dust were unknown.

Second, we did not investigate dietary habits and the use of alcohol among subjects. Also, antioxidants, which have been found to play a protective role against the possible genotoxic effects of chemicals in several studies, were not evaluated.

Third, we did not measure sevoflurane concentration continuously during the operation. Hence, we might have incorrectly estimated its peak value. However, we believe that sevoflurane leakage is greatest at the time of intubation, as was reflected by the value in each hospital.

In conclusion, occupational exposure to sevoflurane seems to induce no significant genotoxicity with the currently prevalent anesthesia management systems.

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