Genotoxicity of inhalation anaesthetics: DNA lesions generated by sevoflurane in vitro and in vivo

Krzysztof Szyfter1,2, Roman Szulc3, Adam Mikstaki, Ireneusz Stachecki3, Małgorzata Rydzanicz1, Piotr Jałoszyński3

1 Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland
2 Department Otolaryngology and Laryngeal Oncology, K. Marcinkowski University of Medical Sciences, Poznań, Poland
3 Department of Anaesthesiology and the Chair of Anaesthesiology and Intensive Therapy, K. Marcinkowski University of Medical Sciences, Poznań, Poland
4 Division of Anaesthesiology and Intensive Care, Regional General Hospital, Poznań, Poland

Abstract. A moderate genotoxic activity of halothane and isoflurane applied as volatile anaesthetics has already been shown. The aim of this work was to estimate a potential genotoxicity of sevoflurane, introduced to clinical practice later than halothane and isoflurane. A genotoxic activity of all three compounds was estimated by using the comet assay in human peripheral blood lymphocytes (PBL) proliferating in vitro. We demonstrated that in contrast to the previously studied anaesthetics, sevoflurane did not induce any increase in DNA migration in the studied conditions. To estimate a genotoxic effect of a prolonged exposure to halogenated anaesthetics in vivo, PBL taken from operating room personnel (n = 29) were tested for DNA degradation and compared with those from a control non-exposed group (n = 20). No significant differences were detected between the groups. We conclude that sevoflurane does not have genotoxic properties, both in vitro and in vivo.

Key words: volatile anaesthetics, genotoxicity, occupational exposure.

Introduction

Human genetic material is challenged by exposure to exogenous and endogenous genotoxic agents. Four main sources of exogenous exposure are associated with environment pollution, hazardous working conditions, nutrition, and life style.

Many occupations are connected with exposure to physical and chemical genotoxic agents. In this respect, the safety of health care workers is discussed primarily in relation to the use of ionising radiation and cytostatic anti-cancer drugs, both capable to induce degradation of genetic material (IARC 1987). Less attention has been paid to simple halogenated hydrocarbons or short-chain ethers used as inhalation anaesthetics in surgery and dentistry (Ferstanding 1995). Halothane [CHClBr-CF3] was the first fluorinated hydrocarbon introduced as an inhalation anaesthetic in 1960s.

The search for new inhaled drugs provided a series of halogenated aliphatic compounds including isoflurane [CF3-CHCl-O-CH2F], sevoflurane [(CF3)2CH-O-CH2F], enfurane, desflurane and others. The first three of them seem to be most common in current clinical practice. Because of the rapid induction and recovery characteristics, lack of pungency, and the agreeable, non-irritant odour, sevoflurane is a preferred anaesthetic agent in ambulatory and nonambulatory surgery in children (Goa et al. 1999).

Effects of inhalation anaesthetics on organs and systems have been widely studied in patients undergoing anaesthesia (Stevens and Kingston 1996). By contrast, the potential health effects of a continuous exposure of operation room personnel to volatile anaesthetics still remain an open question. Health hazards connected with an occupational exposure to inhalation anaesthet-
ics is not sufficiently documented but some reports on nephrocytotoxicity, hepatotoxicity and carcinogenicity have been published (Guirguis et al. 1990; Lucchini et al. 1996). A chronic exposure to halogenated anaesthetics can also affect human reproduction (Boivin 1997). For these reasons halogenated anaesthetics remain under thorough observation of the International Agency for Research on Cancer, Lyon, France (IARC 1987). Taking into account a potential health risk, in the USA the recommended threshold values were set at 25 ppm for N\textsubscript{2}O and 2 ppm for halogenated anaesthetics (Niosh 1977). In EU countries the threshold concentrations of halogenated anaesthetics vary from 2 to 20 ppm (Hoerauf et al. 1997).

In our previous study we have demonstrated genotoxicity of halothane and a weak genotoxic activity of isoflurane in blood lymphocytes proliferating \textit{in vitro}. The genotoxic effect was shown as an increase in DNA single strand breaks in cells exposed to anaesthetics by the alkaline comet assay. Another difference of the effect of the two studied drugs was that halothane-induced DNA fragmentation was not removable due to cell death. Further interpretation was that isoflurane is capable to induce genotoxicity, while halothane is both geno- and cytotoxic (Ja³oszyñski et al. 1999).

The aim of the present study was to compare the \textit{in vitro} genotoxicity of halothane and isoflurane with that of sevoflurane, which has been recently commonly used. The next step was to estimate the \textit{in vivo} genotoxic effect of halogenated anaesthetics in conditions of occupational exposure of hospital staff to volatile anaesthetics, including sevoflurane. The potential genotoxicity was studied as the extent of DNA degradation measured by the comet assay (Ja³oszyñski et al. 1999).

**Material and methods**

**Study subjects**

The group of 29 exposed persons (age: 20-55 years, mean 36.1 ± 9.9 years) was recruited from operating room personnel and included physicians, nurses, and technical anaesthesiology staff. The non-exposed control group consisted of 20 persons (mean age: 32.4 ± 9.4 years). Smoking habits were similar in both groups (21 non-smokers in the exposed group and 11 non-smokers in the control group). Current drug users were not included in the study. The concept of the study was explained to all blood donors who voluntarily agreed to participate in it.

**Lymphocyte isolation and \textit{in vitro} treatment**

Heparinized venous blood samples were collected from healthy non-smoking male volunteers, 24-26 years old. The peripheral blood lymphocyte (PBL) fraction was separated with a standard method by centrifugation over Gradisol L (Polfa, Poland). The viability of cells was higher than 90\%, as examined by trypan blue exclusion. PBL were suspended in RPMI medium 1640 without L-glutamine. Halothane (Leciva, Czech Republic), isoflurane (Abbott Labs, UK) and sevoflurane (Abbott Labs, UK) were dissolved in DMSO directly before treatment. Cells were treated separately with each anaesthetic (concentration of 1 or 10 mM) at 4°C or 37°C. Treatment was carried out for 10 and 30 minutes in sealed glass tubes to avoid evaporation.

Fresh blood samples (5 mL) from the exposed and control subjects were subjected to lymphocyte separation and analysed further by the comet assay.

**Alkaline comet assay**

The protocol already described (Ja³oszyñski et al. 1999) was used without modifications. Image analysis was performed with an AxioPhot fluorescence microscope (Opton, Germany). The length of 50 comets was measured for each experimental point.

**Statistics**

The data were transformed to ranks to estimate the statistical significance by the Mann-Whitney U-test with GraphPadPrism software. Results were considered significant when p < 0.05.

**Results**

All studied volatile anaesthetics were capable to induce a genotoxic effect in human PBL proliferating \textit{in vitro} at 4°C, as shown by an increase in mean comet length by alkaline single-cell electrophoresis (Figure 1). Halothane appeared to be
a stronger genotoxic agent than isoflurane, although the increase in mean comet length induced by each of the two agents was significantly different from the control ($p < 0.05$). A slight genotoxic effect induced by sevoflurane did not reach this level of significance. The experiments performed at $37^\circ$C showed that halothane and isoflurane induced a pronounced genotoxic effect when applied at a high dose (10 mM). An exposure of PBL to halothane, isoflurane or sevoflurane at $37^\circ$C was less effective than at $4^\circ$C. Such a difference can be explained by more efficient processes of detoxication of genotoxicants and removal of DNA breaks in the course of DNA repair at the physiologic temperature. The genotoxic effect was not apparently time-dependent in the studied conditions. Further, it is also worth to point out that an incubation of PBL with 1% DMSO alone, used in the routine procedure as a solvent for anaesthetics, was followed by a measurable decrease in comet length.

An estimation of effects of an exposure of operating room personnel to volatile anaesthetics in vivo was performed by the same technique. The average (means and medians) total comet length for the exposed group was slightly lower than for the control group without any recorded contact with anaesthetics (Table 1). Statistical analysis did not reveal any significant differences between the studied groups, as shown both for means and medians (Table 2).

### Table 1. Average migration ($\mu$M) of PBL DNA from anaesthetics-exposed and non-exposed individuals

<table>
<thead>
<tr>
<th></th>
<th>Exposed</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of persons</td>
<td>29</td>
<td>20</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>41.57 ± 9.00</td>
<td>43.21 ± 8.00</td>
</tr>
<tr>
<td>Median</td>
<td>40.22</td>
<td>43.28</td>
</tr>
</tbody>
</table>

### Table 2. Statistic analysis of comet assay results concerning comparison of genotoxicity of anaesthetics in exposed and non-exposed individuals

<table>
<thead>
<tr>
<th></th>
<th>Exposed (n)</th>
<th>Control (n)</th>
<th>Rank sum (exposed)</th>
<th>Rank sum (control)</th>
<th>U</th>
<th>Z</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Means</td>
<td>29</td>
<td>20</td>
<td>642</td>
<td>633</td>
<td>207</td>
<td>−1.91647</td>
<td>0.055314</td>
</tr>
<tr>
<td>Medians</td>
<td>29</td>
<td>20</td>
<td>679</td>
<td>596</td>
<td>244</td>
<td>−1.18919</td>
<td>0.234373</td>
</tr>
</tbody>
</table>

Discussion

The application of halogenated hydrocarbons and others as anaesthetics goes back to 1956, when halothane was introduced to clinical practice. In search of a better efficiency and tolerance by
patients, the proposals included methoxyflurane, enfurane, isoflurane, sevoflurane and recently desflurane, to list the most common anaesthetic drugs. The studies concerning the safety of hospital staff exposed to volatile anaesthetics provided conflicting results and were reviewed by Stevens and Kingston (1996); Szulc and Szyfter (2001).

A direct cytotoxicity of sevoflurane and isoflurane, at least in relation to liver cells, seems to be excluded in an animal model (Ghantous et al. 1992). However, an application of volatile anaesthetics provides a filtration of inflowing gases through a soda lime to absorb an excess of CO2. Sevoflurane and other halogenated hydrocarbons undergo partial degradation in temperature-dependent manner. A degradation product of sevoflurane denominated as compound A was identified. A comparative study with halogenated anaesthetic drugs passed through soda lime has shown a limited toxicity of halothane and almost undetectable toxicity of isoflurane and sevoflurane at concentrations used in anaesthesia (Strum et al. 1987). Further, Gonsowski et al. (1994) found that compound A did not induce injury to lungs and the small intestine but was capable to cause renal, hepatic and cerebral injury in rats. Studying genotoxic properties of compound A, Morio et al. (1992) excluded its mutagenicity in Ames test. In concordance with this result, Krause et al. (2003) demonstrated a lack of any increase in sister chromatid exchanges (SCEs) in peripheral blood lymphocytes of children after anaesthesia with sevoflurane. On the other hand, rats exposed to halogenated anaesthetics have shown an increased frequency of micronuclei formation in kidney cells. The genotoxic activity of halothane in this model was the highest, followed by a comparable activity of sevoflurane and isoflurane, but no genotoxic activity of enfurane was detected (Robbiano et al. 1998). This is consistent with the results of our earlier study on genotoxicity of halothane and isoflurane, demonstrated in human lymphocytes by the comet assay. In case of halothane, a joined occurrence of geno- and cytotoxicity was established (Ja³oszyñski et al. 1999).

In the present study, halothane and isoflurane were used as a positive control. The genotoxicity of halothane was higher than that of isoflurane. Sevoflurane-induced DNA damage did not differ from the results observed for the DMSO control. Hence, it is concluded that sevoflurane does not exert genotoxic activity in vitro in the studied experimental conditions. A decrease in mean comet length in PBL treated by DMSO used as a solvent for anaesthetics can be explained in two ways: (i) stabilization of the cell wall by DMSO or (ii) inhibitory influence of CYP2E, isoform of cytochrome P450, responsible for activation of sevoflurane and analogous compounds (Kharasch et al. 1995).

Concerning occupational exposure to anaesthetics in Poland, it was already shown that the recommended threshold values for nitrous oxide and halogenated anaesthetics are exceeded, although at the university hospital with the scavenging system it tended to be within EU recommendations (Wiesner et al. 2000). The relatively high exposure to anaesthetics in Polish hospitals seems to be a proper model to study health effects and genotoxicity.

The potential of volatile anaesthetics to exert genotoxic effects in conditions of occupational exposure was studied in several ways. The extended studies on exposure, including contribution of individual drugs, comparison between particular jobs in and outside the operation theatre and significance of protection devices were done by a joint team from Regensburg (Germany) and Vienna (Austria) (Hoerauf et al. 1999, Wiesner et al. 2001). In the course of these studies, Hoerauf et al. (1999) described an induction of SCEs in the group of 27 exposed non-smokers confronted with a relevant non-exposed group. Those authors concluded that genetic damage induced by the exposure to anaesthetics is comparable with that induced by smoking 11-20 cigarettes a day.

Another experimental attempt to estimate a genotoxic potential of volatile anaesthetics was the monitoring of chromosome aberrations in exposed personnel. An increase in chromosome damage was found in three independent studies but in each case authors were not able to exclude the confounding effect of tobacco smoking and X-ray exposure (Lamberti et al. 1989; Rozgaj et al. 2001).

The comet assay has also been applied to detect genotoxicity of volatile anaesthetics. A moderate increase in DNA damage was detected in PBL of patients undergoing anaesthesia with isoflurane or a mixture of halothane and isoflurane (Şardış et al. 1998a; Karabiýik et al. 2001). An anaesthesia-induced genotoxicity was found to be removable within a short time by DNA repair (Karabiýik et al. 2001). Further, operating room personnel (n = 66) exposed to nitrous oxide, halothane and isoflurane has been shown to have a higher DNA migration in PBL. The effect was detected...
both in smokers and non-smokers (Șardaș et al. 1998b).

Altogether, it seems that genotoxicity due to exposure to halogenated anaesthetics is rather weak. Two questions within this topic still remain open: the impact of exposure duration and inter-individual sensitivity related to significance of genetic factors.

Acknowledgements. The study was supported by the State Committee for Scientific Research, grant No. 6 P05C 005 21. The authors are greatly indebted to Prof. H Koroniak and Prof. J Milecki (Faculty of Chemistry, A. Mickiewicz University, Poznań, Poland) for helpful discussions concerning the chemistry and properties of organic fluorinated compounds. A contribution of Dr. R Mikstacka (Chair of Pharmaceutical Biochemistry, Univ. of Med. Sciences, Poznań, Poland) is also appreciated.

REFERENCES


