Genotoxicity of the volatile anaesthetic desflurane in human lymphocytes in vitro, established by comet assay

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Abstract. The aim of the present study was to estimate the genotoxicity of desflurane, applied as a volatile anaesthetic. The potential genotoxicity was determined by the comet assay as the extent of DNA fragmentation in human peripheral blood lymphocytes in vitro. The comet assay detects DNA strand breaks induced directly by genotoxic agents as well as DNA fragmentation due to cell death. Another anaesthetic, halothane, already proved to be a genotoxic agent, was used as a positive control. Both analysed drugs were capable of increasing DNA migration in a dose-dependent manner under experimental conditions applied. The results of the study demonstrated that the genotoxicity of desflurane was comparable with that of halothane. However, considering the pharmacodynamics of both drugs, the genotoxic activity of desflurane may be connected with a less harmful effect on the exposed patients or medical staff.

Key words: comet assay, desflurane, genotoxicity, halothane, inhalation anesthetics.

Introduction

In the current clinical practice many inhalation anaesthetics are in use, including halogenated aliphatic compounds. The oldest in this group is halothane. It was introduced as an anaesthetic in the 1960s. Search for new inhaled drugs has provided a series of halogenated aliphatic compounds, including isoflurane, sevofluirane, enfurane, desflurane and others.

In previous studies, we described genotoxic effects of halothane, isoflurane and sevofluirane (Jaloszyński et al. 1999a, 1999b; Szyfter et al. 2004). Known detrimental effects of volatile anaesthetics are genotoxicity and cytotoxicity (Horeauf et al. 1999; Jaloszyński et al. 1999a; Karabiyik et al. 2001; Lamberti et al. 1989; Rozgaj et al. 2001; Sardaš et al. 1998a, 1998b; Szyfter et al. 2004). The genotoxic effect can be shown by the alkaline comet assay, which measures the induction of DNA single strand breaks in cells exposed to anaesthetics (Jaloszyński et al. 1999a, 1999b; Karabiyik et al. 2001; Möller et al. 2000; Sardaš et al. 1998a, 1998b; Szyfter et al. 2004). DNA strand breaks originate from the direct modification of DNA by chemical agents or their metabolites (Möller et al. 2000; Tice et al. 2000).

The comet assay, known also as the single-cell gel electrophoresis, is commonly used in studies on genotoxicity. As a rapid, simple and inexpensive technique, it has been found particularly useful for preliminary estimation of the genotoxic potential of drugs by evaluation of DNA strand breaks, crosslinks and alkali-labile sites and DNA repair induced by many chemical agents (Möller et al. 2000; Tice et al. 2000; Jaloszyński and Szyfter 1999). In this technique the migration of DNA in an electric field, supposed to be proportional to strand breakage, is a quantitative measure
of genotoxicity (Duez et al. 2003). The genotoxic activity of anaesthetic gases has already been studied by the comet assay (Jałoszyński et al. 1999a; Karabiyik et al. 2001; Ćardaš et al. 1998a, 1998b; Szyfter et al. 2004). Another line of evidence of genotoxic activity of halogenated compounds applied in anaesthesiology comes from numerous cytogenetic observations. Some published reports document an increased number of chromosome aberrations (Karelova et al. 1992; Lamberti et al. 1989; Rozgaj et al. 2001, 1999), increase in micronuclei formation (Robbiano et al. 1998) and a high rate of sister chromatid exchanges (Karelova et al. 1992). However, a lack of genotoxicity of some halogenated anaesthetics has been reported as well (Husum et al. 1983, 1986). Cytogenetic alterations were observed in mammalian cells after in vivo and in vitro exposure to various anaesthetics (Robbiano et al. 1998).

The effects of occupational exposure to inhalation anaesthetics are not sufficiently documented but some reports on nephrotoxicity (Lucchini et al. 1996), hepatotoxicity and carcinogenicity have been published (Robbiano et al. 1998). Some metabolites of anaesthetic gases are associated with toxic effects in certain tissues, e.g. in the liver, kidney or brain (Lucchini et al. 1996; Walker 1996). Anaesthetics can increase the frequency of spontaneous abortion among female operating room personnel or affect human reproduction in other ways (Boivin 1997, Rosenfeld and Loose-Mitchell 1998).

The present work is an extension of the former studies on genotoxicity of halogenated anaesthetics. The goal of this study was to estimate the genotoxicity of desflurane (1,2,2,2-tetrafluoroethyl-difluoromethylether) and to confront it with the already proven genotoxic activity of halothane (2-bromo-2-chloro-1, 1,1-trifluoroethane) (Jałoszyński et al. 1999a; Jałoszyński et al. 1999b; Szyfter et al. 2004). The potential genotoxicity was studied as the extent of DNA degradation measured by the comet assay.

**Material and methods**

**Chemicals**

All chemicals were of analytical grade, purchased from: Serva: normal melting point agarose, Triton X-100 and 4',6-diamidino-2-phenylindol · 2 HCl (DAPI); Sigma: dimethyl sulfoxide (DMSO) and tris; Bio-Rad: low melting point agarose; IITD Poland: RPMI 1640 medium without L-glutamine; Aqua-Medica Poland: Gradisol L; Leciva Czech Republic: halothane (Narcotan); and Baxter Belgium: desflurane (Suprane).

**Peripheral blood lymphocytes: isolation and treatment**

Human peripheral blood lymphocytes (PBL) were obtained from two healthy, 25–27 year old, non-smoking volunteers. The cells were separated by the standard method, including centrifugation over Gradisol L at 1200 rpm for 15 min. The cells were suspended in the RPMI 1640 medium without L-glutamine and exposed to halothane and desflurane at a concentration of 0.1 mM, 1 or 10 mM in 1% DMSO on ice.

Because of a high volatility of both anaesthetics and to prevent repair of induced DNA lesions, the treatment was carried out at 4°C in sealed plastic tubes. After exposure for 5, 10, 30 or 60 min, the cells were settled down by a 10-min spinning (700 rpm) and washed twice with fresh medium. The negative controls were samples with water or with 1% DMSO. The positive controls were samples with halothane. This agent was also used as a positive control in our previous study (Szyfter et al. 2004). Slides were prepared in duplicate.

**Alkaline comet assay**

The alkaline comet assay was conducted as described by Jałoszyński et al. (1999a) and Szyfter et al. (2004). Briefly, the PBL suspension (30 µL) was mixed with 70 µL of 1% low melting point agarose in the RPMI 1640 medium at 37°C. The mixture was pipetted onto microscope slides previously pre-coated with a layer of 1% normal agarose. The slides were immersed in lysis solution (2.5 M NaCl, 0.1 M Na2EDTA, 10 mM tris, 1% of freshly added Triton X-100, pH 10) for 1 h to remove proteins.

The slides were then placed in a horizontal electrophoretic tank in cold buffer (4°C, 3 M NaOH, 1 mM Na2EDTA, pH 13) for 40 min to allow DNA unwinding. The electrophoresis was carried out in the same solution for 30 min (at 300 mA, 0.56 V/cm). After electrophoresis, slides were removed from the tank and immersed in neutralization buffer (0.4 M tris, pH 7.5) and stained with DAPI (2 µg mL⁻¹ in distilled water).

**Image analysis**

Slides were examined with an Axiophot fluorescence microscope (Opton, Germany) with
IMAC-CCD S30 camera and ISIS 3 v 2.00 image analysis system (MetaSystems Hard- and Software, Altlussheim, Germany). The anaesthetic-induced and spontaneous strand breaks were measured as total comet length (increase in DNA migration). Median values were calculated for 100 comets per slide.

Statistics

The data were transformed to estimate the statistical significance by the Mann-Whitney U-test with the use of Graph Pad Prism 4 software. Results were considered significant when p < 0.05.

Results and discussion

The comets resulting from DAPI staining, which visualize the extent of DNA damage, are presented in Figure 1 and 2. They show that desflurane and halothane are capable of inducing DNA fragmentation in human peripheral blood lymphocytes in vitro. Considerable differences between both negative controls (with water and with DMSO) and samples exposed to all concentrations of both anesthetics were observed. DMSO alone was used as an additional negative control because both halothane and desflurane were dissolved in 1% DMSO due to their low solubility in water. The exposure of PBL to halothane and desflurane (Table 1) markedly increased DNA migration in a dose-dependent manner. The increase in mean comet length induced by each of the two drugs was significantly different (p < 0.01) from the control (for halothane $U = 1.000, p = 0.0064$; for desflurane $U = 2.000, p = 0.0092$). The difference between samples treated with halothane and desflurane was not statistically significant ($U = 57, p = 0.4025$).

The analysis of anaesthetic-induced genotoxicity showed that the most harmful was a short (5 minutes) treatment (Figures 3–5). A prolongation of treatment was followed by a tendency to shorten the comet tails but still remaining longer than in water or DMSO experiments. This can be related to DNA repair proceeding during exposure even at low temperature. Another explanation could be the shrinking effect of DMSO towards the cells, which has already been reported. DMSO acts a protective agent and is able to suppress the side effects of anaesthetics during long co-exposure times (Jaloszyński et al. 1999a; Szyfter et al. 2004).

New inhaled anaesthetic agents permit a rapid induction and faster emergence from anaesthesia than halothane. Because these agents are eliminated more rapidly than older drugs, special attention must be paid to postoperative analgesia, as it requires earlier analgesic administration (Walker 1996).

Halothane may result in an unpredictable hepatotoxicity, possibly due to a reactive free radical metabolite. However, incidence of such hepatotoxicity is extremely rare (1 in 10,000–30,000 patients). This drug is also associated with cardiovascular system depression, sensitization of the heart to catecholamines, production of extrasystoles and transient arrhythmias, and relaxation of skeletal muscles. Desflurane is often used in ambulatory surgery. At high doses it decreases peripheral vascular resistance and respiration in a similar way as halothane (Rosenfeld and Loose-Mitchell 1998).

In medical practice the detrimental action of both drugs is different. At least three features,
namely: blood/gas partition coefficient, lipid/gas partition coefficient and percentage of elimination activity of volatile anaesthetics. For inhaled anaesthetic gases, high blood/gas partition coefficients result in a slower elimination. The blood/gas partition coefficient is 2.3 for halothane and 0.42 for desflurane (Hatch 1999; Walker 1996). Lipid/gas partition coefficients describe the solubility in cell lipid membrane and an ability to penetrate into cells. Lipid/gas partition coefficients for desflurane and halothane are 19 and 224, respectively. Therefore, halothane can better penetrate

**Table 1.** Effect of halothane and desflurane on the extent of DNA degradation in peripheral blood lymphocytes, measured by the comet assay (mean comet length ± SD, in µm)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Exposure time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Water</td>
<td>45.07 ± 7.79</td>
</tr>
<tr>
<td>1% DMSO</td>
<td>not determined</td>
</tr>
<tr>
<td>0.1 mM halothane</td>
<td>56.52 ± 8.36</td>
</tr>
<tr>
<td>1 mM halothane</td>
<td>65.37 ± 9.30</td>
</tr>
<tr>
<td>10 mM halothane</td>
<td>67.24 ± 9.30</td>
</tr>
<tr>
<td>0.1 mM desflurane</td>
<td>53.06 ± 9.09</td>
</tr>
<tr>
<td>1 mM desflurane</td>
<td>63.73 ± 10.91</td>
</tr>
<tr>
<td>10 mM desflurane</td>
<td>64.34 ± 8.93</td>
</tr>
</tbody>
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Figure 3. Genotoxic effect of halothane shown in PBL in vitro. A measure of genotoxicity was an induction of DNA migration (µm, median) in comet assay.

Figure 4. Genotoxic effect of desflurane shown in PBL in vitro. A measure of genotoxicity was an induction of DNA migration (µm, median) in comet assay.
into cells, and the difference in solubility may result in a stronger effect on DNA, as compared to desflurane. Metabolism of anaesthetic gases accounts for a small part of their elimination: 20% for halothane, metabolism and 0.02% for desflurane. Desflurane, in contrast to halothane, is metabolized to a minimal degree and the main way of its excretion is through the lungs (Rosenfeld and Loose-Mitchell 1998; Walker 1996).

In our study the effect of both anaesthetics on DNA was similar, although considering their pharmacodynamics halothane should act faster. Probably the published studies disclose a synergistic effect of DMSO with other substances, which was mentioned in some reports (Pommier et al. 1988; Gebel et al. 1999). It is likely that in the present study DMSO could increase the toxic activity of desflurane. However, this is only a supposition.

Hence, the genotoxicity of both studied anaesthetics in vitro is approximately the same. However, considering the published data about the drug’s pharmacodynamics (Rosenfeld and Loose-Mitchell 1998; Walker 1996; Hatch 1999), the toxicity of desflurane for patients seems to be less harmful than that of halothane.

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