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Preliminary study of cytogenetic damage in personnel exposed to anesthetic gases

Ružica Rozgaj[1], Vilena Kašuba[1] and Anamarija Jazbec[2]

[1]Mutagenesis Unit and [2]Biomathematics Unit, Institute for Medical Research and Occupational Health, Ksaverska c.2, HR-10001 Zagreb, Croatia

Occupational exposure to anesthetic gases is associated with various adverse health effects. Genetic material has been shown to be a sensitive target of numerous harmful agents. The aim of this study was to examine whether chromosomal damage could serve to indicate exposure to anesthetics. A group of 43 hospital workers of three professions (anesthesiologists, technicians and operating room nurses) and 26 control subjects were examined for chromosome aberrations, sister chromatid exchanges and micronucleus frequency. The exposed groups matched in duration of exposure to anesthetics, but not in age. An equal ratio between women and men was possible in all groups except nurses. Likewise, the ratio between smokers and non-smokers was also not comparable. An increase in chromosome damage was found in all exposed groups. While the increase in sister chromatid exchange frequency was not significant, chromosome aberrations and micronucleus frequency increased significantly, showing higher rates in women. The results suggest that the micronucleus test is the most sensitive indicator of changes caused by anesthetic gases. The observed difference between sexes with respect to exposure risk call for further, targeted investigations.

Introduction

Occupational exposure to volatile anesthetic agents may result in various adverse health effects. Although eliminated rapidly from the body due to low solubility in blood and tissues, anesthetic gases have been reported to be neurotoxic (Lucchini *et al*., 1996), hepatotoxic (Franco *et al*., 1991) and carcinogenic (Corbett *et al*., 1973). Effects on fertility, an increased incidence of spontaneous abortion and congenital abnormalities have been described (Tannenbaum and Goldberg, 1985; Guirguis *et al*., 1990; Rowland *et al*., 1995). Cytogenetic methods, such as chromosome aberration, sister chromatid exchange and micronucleus determination, have been successfully used in the identification of exposed populations. Numerous studies on exposure to anesthetics have reported genotoxic effects in humans. Reitz *et al*. (1993, 1994) reported the occurrence of DNA single-strand breaks in patients and medical personnel exposed to anesthetic gases. Sardas *et al*. (1998a,b) used the alkaline Comet assay on peripheral blood lymphocytes to assess DNA damage due to anesthetics and found a significant increase in the number of lymphocytes with DNA migration in medical staff exposed to various anesthetic gases, as well as in patients anesthetized with isoflurane. *In vitro* experiments corroborated those results (Jaloszynski *et al*., 1999).

Cytogenetic changes have been observed in mammalian and human cells after *in vivo* and *in vitro* experiments with different anesthetics (Coate *et al*., 1979; Robbiano *et al*., 1998). Of especial concern is long-term occupational exposure to anesthetic gases in hospital workers. The results of studies of chromosome damage in operating room personnel were contradictory. Bigatti *et al*. (1985), Lamberti *et al*. (1989), Natarajan and Santhiya (1990), Karelova *et al*. (1992), Bonassi *et al*. (1997a) and Rozgaj *et al*. (1999) reported an increased frequency of chromosome aberrations (CA). Chang *et al*. (1996) reported an increase in micronucleus (MN) formation, while findings on sister chromatid exchange (SCE) were inconsistent. Husum and Wulf (1980) and Bigatti *et al*. (1985) reported negative results, while Natarajan and Santhiya (1990) and Karelova *et al*. (1992) reported increased SCE frequency in exposed subjects.

Nitrous oxide (N_2O), alone or in combination with other agents such as isoflurane, halothane or enflurane, has become the most common inhalation anesthetic in current anesthesiological practice. The US National Institute of Occupational Safety and Health (NIOSH) has recommended time weighted average concentrations of 50 p.p.m. when N_2O is the sole anesthetic agent and 25 p.p.m. when it is used in conjunction with halogenated agents such as halothane (NIOSH, 1977). Even though artificial ventilation and active scavenging systems are employed in modern hospitals, medical personnel in operating rooms are still exposed to excessive N_2O during various operations, particularly in pediatric anesthesia (Chang *et al*., 1997).

In order to estimate the genotoxic risk of occupational exposure to anesthetic gases as well as to determine the most appropriate test in evaluating that risk, a group of operating room medical workers of different professions were examined by conventional cytogenetic methods: CA analysis, SCE analysis and the MN test.

Materials and methods

Study population

The study involved 69 subjects divided into four groups: controls (26 subjects), anesthesiologists (23 subjects), technicians (8 subjects) and operating room nurses (12 subjects). The exposed subjects were employees in surgical departments of three hospitals. The control subjects were selected from non-exposed workers in the same hospitals among clerks and newly hired employees screened for CA during pre-employment medical tests. All subjects were asked to fill in a questionnaire which included demographic data and data on smoking, use of alcohol, therapeutic drugs, recent vaccination and exposure to radiation therapy. To avoid an influence of ionizing radiation on the results of analyses, we selected staff not exposed to X-rays. According to the answers, all staff worked in operating rooms without ventilation. The most commonly used anesthetics were N_2O and halothane.

Cytogenetic methods

Heparinized blood samples were taken and cultures were initiated within 3 h after collection. One milliliter of whole blood was added to 8 ml of F-10 medium (Gibco) supplemented with 20% calf serum (Biological Industries), antibiotics (100 IU penicillin and 100 µg/ml streptomycin) and 1% phytohem-

[1]To whom correspondence should be addressed. Tel: +385 1 46 73 188; Fax: +385 1 46 73 303; Email: rrozgaj@imi.hr

Table I. Characteristics of groups with respect to sex, age, smoking and years of exposure

	Control	Anesthesiologists	Technicians	Nurses
Sex				
M	15	11	5	
F	11	12	3	12
Age				
Mean ± SD	29.15 (7.5)	42.87 (8.5)	33.50 (7.62)	34.92 (6.1)
Range	20–45	29–58	21–48	21–44
Smoking				
No. smokers (%)	11 (42.3)	8 (34.8)	2 (25.0)	7 (58.3)
No. non-smokers (%)	15 (57.7)	15 (65.2)	8 (75.0)	5 (41.7)
Years of exposure (mean ± SD)		12.91 (8.5)	11.88 (5.4)	11.42 (9.1)

agglutinin (PHA) (Murex). Two replicate cultures for each test were set up from each sample. The cultures were pooled after adding the fixative.

For CA analysis cultures were incubated at 37°C for 48 h. Colchicine was added to the cultures 3 h before harvesting. Giemsa stained slides were coded and scored blind under a light microscope.

For the MN assay 6.0 μg/ml cytochalasin B (Sigma) was added to the cultures at 44 h. Cells were harvested 72 h after PHA stimulation.

Bromodeoxyuridine (Sigma) was added to a final concentration of 10 μg/ml at the start of the cultures for SCE analysis. The cultures were harvested after 72 h cultivation.

Two experienced scorers coded and scored the slides blind for all three methods without karyotyping. The scoring was based on 200 first metaphases per subject for CA, 50 second metaphases per subject for SCE and 1000 binucleated cells per subject for MN.

Statistics

We used Poisson regression to analyze the association between the frequency of CA, SCE and MN as dependent variables and the groups of exposed subjects (anesthesiologists, technicians and nurses), age, sex, smoking status and years of exposure as covariates (Bonassi *et al*., 1997b). The control group served as the baseline. When we found the effect of sex to be statistically significant we regressed the male and female data separately. Poisson regression was done for each type of chromosome aberration and for cells with one, two or three micronuclei. All analyses were performed using SAS 6.12.

Results

Table I shows the distribution of subjects with respect to sex, age, smoking and years of exposure. The exposed groups were matched in duration of exposure to anesthetics, but not in age. The age difference reflects longer training requirements for anesthetists than for nurses and technicians. Similarly, young subjects who were screened during their pre-employment medical tests predominated in the control group. We tried to obtain an equal ratio between women and men in all groups, but this was not possible with nurses. Likewise, the ratio between smokers and non-smokers was also not comparable.

Table II shows the frequencies of CA (single breaks, double breaks, acentric fragments and dicentric chromosomes) and SCE and the frequency and distribution of MN by profession. All types of CA increased in the exposed groups with respect to controls. Chromatid breaks increased only in nurses. SCE frequencies increased slightly in the exposed groups. The frequency of micronucleated cells showed the greatest difference between the exposed groups and controls both in cells with one micronucleus and in those with more micronuclei.

Tables III–V show the results of Poisson regression and include estimated parameters and their standard errors (SE), relative risks (RR), corresponding 95% confidence intervals (CI) and the *P* value.

Table III shows the results of Poisson regression analysis of chromatid breaks, chromosome breaks, acentric fragments and dicentric chromosomes by profession, age, sex, smoking and years of exposure. As all nurses were women, they were treated separately by gender in the regression analysis. The baseline for this regression was females only from the control group. A significantly higher RR was found in nurses for single breaks (RR 3.117), acentric fragments (RR 3.138) and dicentric chromosomes (RR 8.666), while anesthesiologists (RR 2.718) and technicians (RR 3.077) showed significantly higher RR values only for acentric fragments at the $P < 0.05$ level.

Poisson regression of SCEs (Table IV) by profession, age, sex, smoking and years of exposure showed no significant association of SCEs with any of the estimated parameters.

Table V shows Poisson regression analysis of the occurrence of MN. As the differences between sexes were significant with respect to the occurrence of cells with one or two micronuclei, we carried out separate regressions for each sex. Age appeared to influence the RR of MN in men. An increase of 5.5% for each year of age was observed for the frequency of cells containing one MN (RR 1.055). On the other hand, women showed a significantly higher RR for MN, depending on exposure to anesthetics. Female anesthesiologists had an almost twice as high RR for the incidence of cells with one MN and a 7.6 times higher RR for the incidence of cells with two MN than the control group. The increase in cells with three MN was found to be statistically significant in anesthesiologists. The RR for occurrence of cells with three MN in anesthesiologists of both sexes was nearly 27 times higher than in the control group.

Discussion

The results of studies of possible genotoxic effects of anesthetics on occupationally exposed subjects are not always comparable. In addition to anesthetics, medical professionals in operating rooms are exposed to potentially harmful chemicals as well as to X-rays. It is often difficult to distinguish which of these agents caused damage to the genetic material. According to answers in the questionnaire, we selected only those subjects who did not work with any kind of radiation. The increased frequency of aberrations in the exposed subjects in this study confirmed our previous results (Rozgaj *et al*., 1999) and the results of other authors (Bigatti *et al*. 1985; Karelova *et al*., 1992; Bonassi *et al*., 1997a,b).

SCE frequencies were only slightly higher in the exposed groups than in controls (6.89 in doctors, 6.96 in technicians and 6.52 in nurses versus 5.14 in controls) and we did not find that any of the parameters significantly influenced SCE frequency. Similar results were obtained by Lamberti *et al*. (1989), Bigatti *et al*. (1985) and Husum and Wulf (1980),

Table II. Cytogenetic damage in control and exposed subjects by profession

Variable	Control	Doctors	Technicians	Nurses
CA per 200 cells (mean ± SD)				
No. of subjects	26	23	8	12
SB	0.81 ± 0.69	0.65 ± 1.11	0.50 ± 0.76	1.25 ± 1.42
DB	0.30 ± 0.47	0.52 ± 0.95	0.50 ± 0.76	0.67 ± 0.78
AC	0.69 ± 1.26	2.09 ± 1.76	1.88 ± 3.09	2.25 ± 1.71
DIC	0.11 ± 0.43	0.35 ± 0.57	0.38 ± 1.06	0.75 ± 1.22
SCE per cell (mean ± SD)				
SCE	5.14 ± 1.07	6.89 ± 1.75	6.96 ± 2.08	6.52 ± 1.28
MN per 1000 cells (mean ± SD)				
MN 1	7.63 ± 10.21	22.11 ± 14.30	15.00 ± 12.01	23.73 ± 15.32
MN 2	0.64 ± 1.68	4.74 ± 6.51	2.25 ± 2.05	1.18 ± 1.47
MN 3	0.09 ± 0.29	0.63 ± 1.16	0.50 ± 0.93	0.18 ± 0.60
MN 4	0	0.11 ± 0.32	0.25 ± 0.46	0.09 ± 0.30

SB, chromatid break; DB, chromosome break; AC, acentric fragment; DIC, dicentric chromosome; MN 1–4, binucleated lymphocytes containing one, two, three or four micronuclei.

Table III. Estimated parameters, RR and 95% confidence intervals estimated by Poisson regression for CA as dependent variable

Variable	Parameter estimate	SE	*P*	RR	95% CI
Chromatid break					
Anesthesiologists	0.4529	0.4136	0.2734	1.573	0.69–3.59
Technicians	0.3364	0.6168	0.5855	1.399	0.40–4.79
Nurses	1.1371	0.4314	0.0084	3.117	1.32–7.37
Age	0.0202	0.0244	0.4065	1.020	0.97–1.07
Sex	–0.0654	0.0359	0.8498	0.936	0.87–1.01
Smoking	–0.2579	0.2827	0.3616	0.772	0.44–1.36
Years of exposure	–0.0823	0.0302	0.0065	0.921	0.87–0.98
Chromosome break					
Anesthesiologists	0.2276	0.5969	0.7029	1.255	0.37–4.13
Technicians	0.4619	0.7151	0.4172	1.587	0.38–6.61
Nurses	0.7491	0.6840	0.2735	2.115	0.54–8.28
Age	0.0291	0.0347	0.4029	1.029	0.96–1.10
Sex	–0.1912	0.4379	0.6624	0.825	0.34–1.97
Smoking	0.2459	0.3738	0.5108	1.278	0.61–2.70
Years of exposure	–0.0057	0.0373	0.8793	0.994	0.92–1.07
Acentric fragments					
Anesthesiologists	1.0001	0.3288	0.0024	2.718	1.41–5.24
Technicians	1.1241	0.4048	0.0055	3.077	1.37–6.90
Nurses	1.1436	0.3824	0.0028	3.138	1.46–6.73
Age	0.0231	0.0199	0.2440	1.023	0.98–1.06
Sex	0.1053	0.2319	0.6497	1.111	0.69–1.76
Smoking	0.1691	0.2068	0.4134	1.184	0.78–1.79
Years of exposure	–0.0146	0.0206	0.4778	0.985	0.94–1.03
Dicentric chromosomes					
Anesthesiologists	1.1241	0.7892	0.1543	3.077	0.64–14.86
Technicians	1.3824	0.9050	0.1266	3.984	0.65–24.24
Nurses	2.1595	0.8176	0.0083	8.666	1.69–44.30
Age	0.0258	0.0425	0.5432	1.026	0.94–1.11
Sex	–0.2150	0.5554	0.6987	0.806	0.26–2.44
Smoking	–0.0246	0.4412	0.9556	0.975	0.40–2.35
Years of exposure	–0.0276	0.0405	0.4955	0.972	0.89–1.05

Relative risks for the exposed groups are estimated relative to the control group. Bold indicates statistically significant values at $P < 0.05$ level.

while Sardas *et al*. (1992) and Karelova *et al*. (1992) reported a significant increase in SCE frequency in medical workers exposed to volatile anesthetics. Natarajan and Santhiya (1990) found an increase in SCEs in medical pesonnel exposed to anesthetics, although it was not significant.

MN were the most appropriate biomarker of anesthetic genotoxicity to humans. The number of cells containing one or more MN increased in all exposed groups, which was in agreement with other authors. Chang *et al*. (1996) reported increased MN formation in nurses occupationally exposed to N_2O. Robbiano *et al*. (1998) tested six halogenated anesthetics for their ability to induce MN formation in rat kidney. All except enflurane significantly increased the MN frequency, while halothane and trichloroethylene also reduced binucleated cells, presumably due to toxicity.

Sex proved to be one of the factors influencing MN frequencies in human lymphocytes (Bolognesi *et al*., 1997). A difference in MN frequency between sexes in our study was also evident, with higher values in exposed women. Fenech *et al*. (1994) reported a significantly higher spontaneous MN

Table IV. Estimated parameters, RR and 95% confidence intervals estimated by Poisson regression for SCE as dependent variable

Variable	Parameter estimate	SE	*P*	RR	95% CI
Anesthesiologists	0.3124	0.1634	0.0559	1.367	0.98–1.89
Technicians	0.3653	0.2046	0.0741	1.441	0.96–2.17
Nurses	0.2462	0.1976	0.2126	1.279	0.86–1.89
Age	0.0033	0.0101	0.7467	1.003	0.98–1.02
Sex	0.0038	0.1263	0.9758	1.004	0.78–1.29
Smoking	0.1022	0.1113	0.3583	1.107	0.89–1.38
Years of exposure	–0.0039	0.0114	0.7345	0.996	0.97–1.02

Table V. Estimated parameters, RR and 95% confidence intervals estimated by Poisson regression for MN as dependent variable

Variable	Parameter estimate	SE	*P*	RR	95% CI
MN1					
Male					
Anesthesiologists	0.3176	0.3106	0.3065	1.374	0.72–2.60
Technicians	0.1964	0.2518	0.4354	1.217	0.72–2.04
Nurses					
Age	0.0541	0.0165	0.0011	1.055	1.02–1.09
Smoking	–0.2690	0.1208	0.0259	0.764	0.59–0.98
Years of exposure	–0.0116	0.0200	0.5620	0.988	0.95–1.03
Female					
Anesthesiologists	0.6515	0.1410	0.0001	1.918	1.44–2.56
Technicians	0.8390	0.1909	0.0001	2.314	1.57–3.41
Nurses	0.8664	0.1545	0.0001	2.378	1.74–3.26
Age	0.0110	0.0087	0.2035	1.011	0.99–1.03
Smoking	–0.0353	0.0901	0.6953	0.965	0.80–1.16
Years of exposure	0.0010	0.0080	0.9007	1.001	0.98–1.02
MN2					
Male					
Anesthesiologists	1.3538	0.9415	0.1505	3.872	0.56–26.82
Technicians	1.4537	0.7698	0.0590	4.280	0.88–20.82
Nurses					
Age	0.0643	0.0503	0.2008	1.066	0.96–1.18
Smoking	–1.3295	0.4652	0.0043	0.264	0.10–0.68
Years of exposure	–0.0751	0.0665	0.2578	0.927	0.81–1.06
Female					
Anesthesiologists	2.0280	0.4320	0.0001	7.598	3.15–18.32
Technicians	1.1635	0.6029	0.0536	3.201	0.94–10.93
Nurses	0.3630	0.5625	0.5187	1.437	0.45–4.52
Age	–0.0097	0.0304	0.7507	0.990	0.93–1.05
Smoking	–0.4521	0.2634	0.0861	0.636	0.37–1.08
Years of exposure	0.0092	0.0282	0.7437	1.009	0.95–1.07
MN3					
Male + female					
Anesthesiologists	3.3160	1.2215	0.0066	27.549	2.39–317.42
Technicians	1.6379	1.0173	0.1074	5.144	0.67–39.39
Nurses	1.8083	1.3330	0.1749	6.100	0.42–87.32
Age	–0.1198	0.0750	0.1099	0.887	0.76–1.03
Sex	–0.9617	0.5853	0.1004	0.382	0.12–1.23
Smoking	–1.9358	0.7627	0.0111	0.144	0.03–0.66
Years of exposure	0.0768	0.0729	0.2923	1.079	0.93–1.25

Relative risks for the exposed groups are estimated relative to the control group. Bold indicates statistically significant values at $P < 0.05$ level.

frequency in women and suggested as a possible cause loss of X chromosomes, which then contributed to MN yield in women. Bonassi *et al*. (1995) confirmed the increase in MN rate in women and no difference between sexes in SCE or CA frequency. In contrast, in a study of healthy donors Di Giorgio *et al*. (1994) did not find any influence of sex on MN level.

Age- and smoking-related effects on MN occurrence were found in men, but not in women, in our study. On the other hand, the effect of exposure to anesthetics was significant in women and not in men. Significantly higher RR values for MN in women and significantly increased RR values for CAs in nurses could indicate a higher sensitivity to anesthetics in women. It is well known that MN arise from chromosome breakage or chromosome loss. To increase the sensitivity of the test, e.g. to distinguish clastogenic from aneugenic effects, a combination of the MN assay with fluorescence *in situ* hybridization should be used (Kirschvolders *et al*., 1997; Elhajouji *et al*., 1998).

Recent findings suggest that in addition to the influence of age and sex, dietary factors affect chromosome aberrations and MN frequency. Exposure to N_2O increases the plasma levels of folate and homocysteine and decreases plasma levels

of methionine (Ermens *et al*., 1991; Koblin and Evermann, 1991). N_2O destroys vitamin B12, which in turn leads to an increase in MN frequency. This could be an explanation for the increase in MN in personnel exposed to N_2O. Fenech (1999) reported that MN frequency was minimized when plasma homocysteine was <7.5 μmol/l and plasma B12 was >300 pmol/l. It is, therefore, important to take into account the influence of plasma B12 and homocysteine status when using the MN assay for human biomonitoring studies.

In conclusion, our study singled out the MN test as the most appropriate for evaluating genotoxic effects of anesthetic gases on humans. Of special concern is the likely higher risk for women. The results presented here are only preliminary, due to a small control group which was not tightly matched to the exposed groups for all characteristics. Further study should include a better design with optimal selection of controls and exposed groups tightly matched for age, gender, smoking and plasma levels of folate and vitamin B12. As data on the relation between sex and occurrence of MN are mostly limited to the general population, a targeted investigation should include a larger group of medical professionals exposed to anesthetics.

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