Anesthesiology
62:475–479, 1985

Monitoring of Sister Chromatid Exchanges in Lymphocytes of Nurse-Anesthetists

Bent Husum, M.D., Hans Christian Wulf, M.D., Ph.D.,† Erik Niebuhr, M.D., Ph.D.‡*

Cytogenetic methods are used increasingly for monitoring exposure to potential mutagens/carcinogens in the environment. By one such method, the sister chromatid exchange (SCE) test, comparison of different groups of hospital personnel has not indicated any mutagenic effect of occupational exposure to waste anesthetic gases. Since no information is available on repeated examinations of operating room personnel during a longer period of occupational exposure, the authors examined SCE in lymphocytes in a total number of 191 venous blood samples drawn from 14 previously unexposed nurses before and during up to 32 months of training as nurse-anesthetists. The initial SCE/cell ranged from 8.03 to 13.13 SCE/cell. Individual linear regressions were performed for the transformed variable, $y = (\text{sum SCE})^{1/2} + (\text{sum SCE} + 1)^{1/2}$, on time; and for the first 6-month period, the weighted mean of individual slopes was $b_0 = -0.119 \pm 0.088$, not significantly different from zero. Calculated for the whole observation period, $b_0 = 0.030 \pm 0.014$, $P = 0.034$ (two-tailed *t* test). Converted into SCE/cell, SCE would decrease 0.10 SCE/cell for each 6-month period of exposure (95% confidence limits 0.07–0.13 SCE/cell). The reason for this apparent decrease remains unknown. The results of the present study were in accord with previous studies of operating room personnel and of patients anesthetized with inhaled anesthetics. It was concluded that there is no indication, from the SCE test, of a mutagenic action due to exposure *in vivo* to currently used inhalation anesthetics. (Key words: Anesthetics, gases: nitrous oxide. Anesthetics, volatile: enflurane; halothane. Operating rooms: contamination. Toxicity: anesthetics; mutagenicity.)

CYTOGENETIC METHODS are used increasingly for monitoring exposure to potential mutagens/carcinogens in the environment.[1] In such studies of possible genotoxic hazards associated with occupational exposure to waste anesthetic gases, examination of sister chromatid exchanges (SCE) in peripheral lymphocytes have revealed no indication of a mutagenic effect from long-term exposure to trace concentrations of inhalation anesthetics.[2–5] Since these reports were all based on comparison of groups of exposed and nonexposed persons and each person was examined only once, no information is available on repeated examinations of operating room personnel during a longer period of occupational exposure. Therefore, we examined SCE in lymphocytes in a total of 191 venous blood samples drawn from 14 nurses before and during up to 32 months of training as nurse-anesthetists.

Materials and Methods

The study was performed on 14 nurses (13 women and one man) coming to the Department of Anesthesia, Rigshospitalet, Copenhagen, in order to receive a 2-year training in anesthesia. All the nurses were in good health, in general, and none received regular medication, except in some cases oral contraceptives. The pertinent personal data for each of the nurses are shown in table 1. All of the nurses had completed their basic education and were registered nurses, and none of them previously had worked in anesthesia. Before they stated working in the department, the nurses were informed about the possible health hazard associated with working in an environment contaminated by waste anesthetic gases; they also were instructed on the significance of careful working procedures with regard to minimizing the exposure levels. The nurses all consented to participate in the study.

The 2-year training program included a 3-month stay in each of eight different units, so that the nurses were exposed to all types of anesthetic practice. The inhalation anesthetics used in the department included halothane, enflurane, and nitrous oxide. In all operating theaters the anesthetic circuits were equipped with scavenging systems for removal of waste anesthetic gases. No attempt was made to monitor the exposure levels to which the trainees actually were exposed. However, periodic routine measurements of the concentrations of halothane and nitrous oxide in the breathing zones of experienced nurse anesthetists indicated that these nurses were able to maintain concentrations below the Danish limits for time-weighted averages of 5 ppm for halothane and 100 ppm for nitrous oxide.

* Senior Registrar, Department of Anesthesia, Rigshospitalet (University Hospital), Blegdamsvej 9, DK-2100, Copenhagen, Denmark.

† Senior Registrar, Department of Dermatology, The Finsen Institute, Copenhagen, Denmark.

‡ Assistant Professor, The University Institute of Medical Genetics, Copenhagen, Denmark.

Received from the Department of Anesthesia, Rigshospitalet (University Hospital), Blegdamsvej 9, Copenhagen, Denmark, the Department of Dermatology, The Finsen Institute, Copenhagen, Denmark, and The University Institute of Medical Genetics, Copenhagen, Denmark. Accepted for publication October 11, 1984.

Address reprint requests to Dr. Husum: Department of Anesthesia, Rigshospitalet (University Hospital), Blegdamsvej 9, DK-2100 Copenhagen, Denmark.

Sampling of venous blood was performed before the nurses started working in the department, during the first 6-month period with intervals of 1–2 weeks and with intervals of 1–3 months thereafter during a total period of up to 32 months. The blood samples were labeled with code numbers, so that the investigators were unaware of the time of withdrawal.

Analysis of SCE was performed by means of a standardized technique[6]: A 0.5-ml aliquot of blood from each sample was incubated in 7.5 ml of Parker 199 standard medium with 1.5 ml of fetal calf serum, 0.2 ml of phytohaemagglutinin (Wellcome, England) and bromdeoxyuridine (BrdU) 2×10^{-5} mol·l^{-1} (6 μg·ml^{-1}). The cells were grown in the dark at 37° C for 72 h, and during the last 2 h Colcemid® was added to the medium to a final concentration of 3×10^{-7} mol·l^{-1}. After treatment with hypotonic potassium chloride 75 mmol·l^{-1} and fixation with glacial acetic acid in methanol (1:3), slides were prepared. After air-drying, the slides were treated with bisbenzimide and ultraviolet light and stained with Giemsa. This made old and newly synthetized DNA material color differently.

A total of 191 successful cultures were obtained. Thirty metaphases were scored for SCE in each specimen, one SCE being counted each time two adjacent segments of one of the chromatids in a chromosome were stained differently.

Statistical Methods

The number of SCE in different cells of an individual person was assumed to behave as coming from a mixture of Poisson distributions.[7] Therefore, the sum of SCE in 30 cells from each person followed a Poisson distribution, and the transformation $y = (\text{sum SCE})^{1/2} + (\text{sum SCE} + 1)^{1/2}$ produced a normally distributed variable, y, by which possible intrapersonal variations throughout the observation period might be evaluated.[7]

For each of the 14 persons was calculated the linear regression of the variable, y, on time, both for the first 6-month period and for the whole observation time. The parallelism of the individual regression lines was tested by comparing the variation between slopes with the variation within individual sets, *i.e.*, by calculation of the variance ratio.[8] The weighted means of individual slopes were tested against zero by a two-tailed *t* test. A significance level of *P* less than 0.05 was used.

Results

Three of the nurses left the department after 4, 4, and 15 months, respectively. In order to avoid losing any relevant information, the data on these three persons were not excluded from the statistical evaluation. From the remaining 11 nurses, a total of 172 successful cultures were obtained during individual observation periods of 24–32 months.

TABLE 1. Personal Data on 14 Nurses in Whom Sister Chromatid Exchanges Were Examined before and during up to 32 Months of Training as Nurse-anesthetists

Subject Number	Sex	Age (yr)	Smoking (daily consumption)	Observation Time (months)	No of SCE Analyses
1	F	26	10–15 cig.	24	17
2	F	28	0	24	21
3	F	30	0	24	20
4	F	32	0	4	7
5	F	27	15 cig.	24	16
6	F	25	0	4	4
7	F	28	20 cig.	32	16
8	F	31	0	32	13
9	F	32	15–20 cig.	15	8
10	F	29	0	29	17
11	M	26	5 g pipe tobacco	24	13
12	F	27	20–30 cig.	30	15
13	F	26	1 cig.	28	12
14	F	26	0	24	12

The initial SCE/cell ranged from 8.03 to 13.13 SCE/cell (mean 9.34 SCE/cell). The individual slopes of regression on time of the transformed variable, $y = (\text{sum SCE})^{1/2} + (\text{sum SCE} + 1)^{1/2}$, ranged from −0.5370 to 0.4756 in the first 6-month period and from −0.1169 to 0.0259 in the whole observation period (table 2).

Testing for parallelism of the individual slopes by comparison of the residual variances "within" the subjects with the variance "between" subjects, indicated that the individual slopes were not significantly different (table 3).

TABLE 2. Individual Slopes of Regression on Time (months) of the Transformed Variable $y = (\text{sum SCE})^{1/2} + (\text{sum SCE} + 1)^{1/2}$. Regressions Are Calculated for the First Six-month Period and for the Whole Observation Period (all samples)

Subject Number	Time (Months)	Initial SCE/cell	First Six Months		All Samples	
			n	Slope	n	Slope
1	24	8.83	10	0.1134	17	0.0251
2	24	8.57	10	0.4513	21	0.0259
3	24	8.03	10	−0.0190	20	−0.0146
4	4	9.43	7	0.0287		
5	24	10.30	9	−0.2108	16	−0.1091
6	4	8.17	4	0.4756		
7	32	9.50	7	−0.4843	16	−0.0266
8	32	9.87	6	−0.1884	13	−0.0664
9	15	8.79	5	−0.4799	8	−0.1169
10	29	8.80	8	−0.2005	17	−0.0011
11	24	10.27	7	−0.5370	13	−0.0646
12	30	13.13	7	−0.4702	15	−0.0628
13	28	8.43	3	−0.4987	12	0.0244
14	24	8.63	3	0.0617	12	−0.0514

n = Number of SCE analyses.

TABLE 3. Statistical Evaluation of Linear Regressions of the Transformed Variable, y = (sum SCE)$^{1/2}$ + (sum SCE + 1)$^{1/2}$, on Observation Time in Months

	First Six-month Period (n = 96)	Whole Observation Period (n = 180)
Range of individual slopes	−0.5370–0.4756	−0.1169–0.0259
Combined individual variances, s_1^2	2.671 (df = 68)	2.944 (df = 156)
Variance between slopes, s_2^2	2.812 (df = 13)	2.204 (df = 11)
Variance ratio, $V^2 = s_2^2/s_1^2$	1.053 (df = 13.68)	0.749 (df = 11.156)
Weighted mean of individual slopes, b_0	−1.119	−0.030
Combined estimate of s_1^2 and s_2^2	2.690 (df = 81)	2.900 (df = 167)
Standard deviation of b_0, sd (b_0)	0.088	0.014
P value, two-tailed *t* test	NS	0.034

n = number of SCE analyses.

For the first 6-month period, the weighted mean of individual slopes, b_0, with the weighted standard deviation, sd(b_0), was -0.119 ± 0.088, not significantly different from zero (table 3). The corresponding values based on the whole observation period were $b_0 = -0.030 \pm 0.014$, $P = 0.034$ (two-tailed), indicating a small, statistically significant decrease of y during the observation period. Converted into SCE/cell, this meant that from an initial value of 9.34 SCE/cell, SCE would decrease 0.10 SCE/cell for each 6-month period of exposure (95% confidence limits 0.07–0.13 SCE/cell).

Discussion

The possibility of a health hazard in anesthetic practice has stimulated extensive discussion during the last 15 yr (reviews in Cohen[9] and Cottrell[10]). Experimental and epidemiologic studies indirectly have indicated that working in an environment contaminated by waste anesthetic gases may be associated with an increased risk of miscarriage, congenital malformations in offspring, and cancer. A causal relationship has never been proved, and rather than exposure to anesthetic gases, some sort of "stress" has been implicated by some as the probable cause of any health problems.[11]

Laboratory tests using changes in the DNA material as an indicator of potential mutagenicity/carcinogenicity are used increasingly to evaluate chemicals in the environment, and inhalation anesthetics have been studied by several such tests. The most widely used mutagenicity test, the Ames Salmonella bacterial assay,[12] has not indicated any mutagenic action of currently used anesthetics such as halothane,[13,14] enflurane,[14,15] or isoflurane,[14,15] whereas vinyl-containing anesthetics such as fluroxene gave positive responses in the presence of a rat liver enzyme system.[15]

Examination of SCE is used increasingly in mutagenicity tests comprising mammalian cells. SCE involves an exchange of DNA segments between the two sister chromatids in a chromosome during cell replication, and although the molecular mechanism leading to SCE formation is not fully understood, it is believed to reflect damage and/or repair of the DNA material. An increased frequency of SCE is an indicator of exposure to mutagenic/carcinogenic substances.[16]

In a thorough study by White *et al.*,[17] examination of SCE in Chinese hamster ovary (CHO) cells following exposure to 10 inhalation anesthetics *in vitro* indicated, in accord with the results from the Ames test,[13–15] that only the vinyl-containing anesthetics were mutagenic, and only so in the presence of enzymes prepared from the livers of Aroclor 1254-pretreated rodents. Therefore, metabolism of the vinyl groups with formation of epoxides was thought to be the cause of the positive mutagenicity of the vinyl-containing compounds.[17] In humans, unlike other species, fluroxene primarily is biotransformed to trifluoroacetic acid, which is believed to be without serious toxic effects.[18]

Because metabolites may be mutagens/carcinogens, and because biotransformation may be different in different species, examination of SCE is performed increasingly on lymphocytes from peripheral blood obtained from humans exposed *in vivo*. Possible environmental cytogenetic hazards have been investigated by this technique in a number of professions, and increased frequencies of SCE have been demonstrated, *e.g.*, in nurses handling cytostatic agents,[3,19] in persons using organic solvents in laboratories,[20] and in sterilization workers who handle ethylene dioxide.[1]

Increased SCE in lymphocytes has been demonstrated as early as 75 min after the administration of a known SCE-inducing agent,[7] and an increased level of SCE following an acute exposure is believed to persist for at least 4–16 weeks.[21]

In a previous study of SCE in operating room personnel,[5] there was no difference between SCE in lymphocytes from anesthetists who had worked from 18 to 312 months (median 8.5 yr) in the operating room and from

unexposed control persons. The present study is, to our knowledge, the first in which SCE has been examined repeatedly during a period of occupational exposure. In this way the individual persons served as their own control, and by continuing the observation for up to 32 months, we should have been able to detect any significant change of SCE that might appear during the period of occupational exposure.

The variance of the "spontaneous" SCE count within subjects exposed to no known mutagens previously has been estimated to be $s_0^2 = 2.64$.[22] This estimate does not differ from the values observed in the present study, which therefore may be explained exclusively as the variance associated with the experiment *per se*. Thus, there was no indication of a variance component that might be associated with the working conditions of the subjects, and this was the case both among smokers and nonsmokers.

The weighted mean of individual slopes of the regression lines was not significantly different from zero in the first 6-month period. When calculated for the whole observation period, there was a small but statistically significant decrease of SCE during the period ($P = 0.034$). We do not know the reason for this. We have no reason to believe that the explanation could be instability of the SCE analysis and drift of the SCE level during the study period.

Nothing is known about a possible role of "stress" on the subjects in altering SCE monitoring. Since some exposure to inhalation anesthetics is almost inevitable when working in the operating room, we have focused on this aspect of the working conditions. From studies of SCE in healthy cigarette smokers, it is known that SCE increases linearly in proportion to the daily cigarette consumption,[23] and this reflects that it is possible to detect even small increases of SCE and their association with low levels of exposure. Assuming that the SCE test is adequate for detection of a possible mutagenic action of even very low levels of exposure to anesthetic gases, our results indicate that inhalation anesthetics do not exert such an action in humans. In favor of this are also the results of previous investigations of patients undergoing anesthesia with either halothane,[24] enflurane,[24], fluroxene,[25] or isoflurane,[26] following which SCE did not change significantly for up to 5 days thereafter.

Summarizing our present knowledge, it appears that by examination of SCE in peripheral lymphocytes after exposure *in vivo*, 1) nurse-anesthetists with a median duration of employment of 8.5 yr are not different from unexposed controls[5]; 2) nurse-anesthetists examined before and during up to 32 months of working in the operating room do not have increased SCE; and 3) in patients exposed to anesthetic concentrations of currently used inhalation anesthetics, SCE is not increased.[4,24,26]

On the basis of these findings, we conclude that there is no indication, from the SCE test, of a mutagenic action due to exposure *in vivo* to currently used inhalation anesthetics.

The statistical evaluation was performed by H. Engberg-Pedersen, Department of Mathematics and Statistics, Leo Pharmaceutical Products, Copenhagen. Technical help in the laboratory was given by Anita Niebuhr and Helle Jensen, and secretarial help was given by Birgitte Neergaard.

References

1. Lambert B, Lindblad A, Holmberg K, Fransesconi D: The use of sister chromatid exchange to monitor human populations for exposure to toxicologically harmful agents, Sister Chromatid Exchange. Edited by Wolff S. New York, John Wiley and Sons, 1982, pp 149–153
2. Husum B, Wulf HC: Sister Chromatid exchanges in lymphocytes in operating room personnel. Acta Anaesthesiol Scand 24:22–24, 1980
3. Waksvik H, Klepp O, Brøgger A: Chromosome analyses of nurses handling cytostatic agents. Cancer Treat Rep 65:607–610, 1981
4. Holmberg K, Lambert B, Lindsten J, Söderhäll S: DNA and chromosome alterations in lymphocytes of operating room personnel and in patients before and after inhalation anaesthesia. Acta Anaesthesiol Scand 26:531–539, 1982
5. Husum B, Niebuhr E, Wulf HC, Nørgaard I: Sister chromatid exchanges and structural chromosome aberrations in lymphocytes in operating room personnel. Acta Anaesthesiol Scand 27:262–265, 1983
6. Wulf HC: Modified method for demonstrating sister chromatid exchange (SCE). Dan Med Bull 27:35–37, 1980
7. Wulf HC, Husum B, Plesner AM, Niebuhr E: Distribution of SCEs in lymphocytes in persons with normal, slightly increased, and heavily increased SCEs. Mutat Res 125:263–268, 1984
8. Hald A: Statistical Theory with Engineering Applications. New York, John Wiley and Sons, 1952, pp 579–584
9. Cohen EN: Anesthetic Exposure in the Workplace. Littleton, PSG Publishing, 1980
10. Cottrell JE: Occupational hazards to the operating room and recovery room personnel. Int Anesthesiol Clin 19:39–183, 1981
11. Vessey MP: Epidemiological studies of the occupational hazards of anaesthesia—a review. Anaesthesia 33:430–438, 1978
12. Ames, BN, McCann J, Yamasaki E: Methods for detecting carcinogens and mutagens with the salmonella/mammalian-microsome mutagenicity test. Mutat Res 31:347–364, 1975
13. Baden JM, Brinkenhoff M, Wharton RS, Hitt BA, Simmon VF, Mazze RI: Mutagenicity of volatile anesthetics: Halothane. ANESTHESIOLOGY 45:311–318, 1976
14. Waskell L. A study of the mutagenicity of anesthetics and their metabolites. Mutat Res 57:141–153, 1978
15. Baden JM, Kelley M, Wharton RS, Hitt BA, Simmon VF, Mazze RI: Mutagenicity of halogenated ether anesthetics. ANESTHESIOLOGY 46:346–350, 1977
16. Perry P, Evans HJ: Cytological detection of mutagen–carcinogen exposure by sister chromatid exchange. Nature 258:121–125, 1975

17. White AE, Takehisa S, Eger EI, Wolff S, Stevens WC: Sister chromatid exchanges induced by inhaled anesthetics. ANESTHESIOLOGY 50:426–430, 1979
18. Johnston RR, Cromwell TH, Eger EI, Cullen D, Stevens WC, Joas T: The toxicity of fluroxene in animals and man. ANESTHESIOLOGY 38:313–319, 1973
19. Norppa H, Sorsa M, Vainio H, Gröhn P, Heinonen E, Holsti L, Nordman E: Increased sister chromatid exchange frequencies in lymphocytes of nurses handling cytostatic drugs. Scand J Work Environ Health 6:299–301, 1980
20. Funes-Cravioto F, Kolmodin-Hedman B, Lindsten J, Nordenskjöld M, Zapata-Gayon C, Lambert B, Norberg E, Olin R, Swensson A: Chromosome aberrations and sister-chromatid exchange in workers in chemical laboratories and a rotoprinting factory and in children of women laboratory workers. Lancet 13:322–325, 1977
21. Vainio H, Sorsa M, Rantanen J, Hemminki K, Aitio A: Biological monitoring in the identification of the cancer risk of individuals exposed to chemical carcinogens. Scand J Work Environ Health 7:241–251, 1981
22. Wulf HC, Husum B, Engberg-Pedersen H, Niebuhr E: Guidelines for the statistical evaluation of SCE, Sister Chromatid Exchange: 25 years of Experimental Research. Edited by Tice RR, Hollaender A. New York, Plenum Press, 1984 (In press)
23. Husum B, Wulf HC, Niebuhr E: Increased sister chromatid exchange frequency in lymphocytes in healthy cigarette smokers. Hereditas 96:85–88, 1982
24. Husum B, Wulf HC, Niebuhr E: Sister chromatid exchanges in lymphocytes after anaesthesia with halothane or enflurane. Acta Anaesthesiol Scand 25:97–98, 1981
25. Husum B, Wulf HC, Niebuhr E: Sister chromatid exchanges in human lymphocytes after anaesthesia with fluroxene. Br J Anaesth 54:987–990, 1982
26. Husum B, Wulf HC, Niebuhr E, Kyst A, Valentin N: Sister chromatid exchanges in lymphocytes of humans anaesthetized with isoflurane. Br J Anaesth 56:559–564, 1984