The Incidence of Chromosomal Aberrations in Anaesthetists

Flossie Jayakaran and I. Manorama Thomas

Division of Human Genetics, Department of Anatomy, St. John's Medical College, Bangalore, India

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Summary Anaesthetists have reported complaints of headache, nausea, fatigue, dizziness, as well as heart and liver diseases as a consequence of chronic exposure to low ambient levels of anaesthetic gases in the operation theater. Reports of foetal wastage, bad obstetric history and infertility have also been recorded. This study was aimed at evaluating the damage to chromosomes by quantifying the damage in terms of the number of chromosomal aberrations (CAs) seen when compared to equally matched controls. The study showed that the anaesthetists showed a significantly higher number of chromosomal aberrations than did the controls. This is evidence of genotoxicity, and suggestions are made to reduce the exposure in the theater by the installation of efficient scavenging systems and leak proof ventilation systems on a top priority basis.

Key words Chromosomal aberrations, Volatile anaesthetics, Halothane, Genotoxic.

Anaesthesia is a regular feature in all surgical interventions. Attempts to keep the operation theater (OT) sterile, leads to an accumulation of waste anaesthetic gases (AGs), escaping from the anaesthetic apparatus as well as exhalations from the anaesthetized patients. With the passage of time and in the absence of scavenging systems, there is a significant build-up of the gas levels in the theater.

Various complaints from OT personnel have been attributed to chronic inhalation during surgery of the ambient AGs in the theater. The AG levels are also higher in the area immediately surrounding the anaesthetist (Spierdijk and Burm 1982).

Epidemiological studies have ascribed adverse reactions by anaesthetists as a consequence of exposure. These reported adverse effects range from feelings of discomfort, headache, nervousness, sleep loss, fatigue, pruritus, increased cancer rate, hepatic and renal diseases to reports of infertility, increased rates of abortions, miscarriages and congenital abnormalities in children born to practicing anaesthetists, as well as a sex ratio favouring the female sex. The aim of the present work was to investigate the toxicity of AGs on the chromosomes of anaesthetists for the incidence of chromosomal aberrations (CAs) like breaks, gaps, rings, dicentrics and exchanges. CAs are very sensitive indicators of mutagenicity and the residual aberrations left behind (after normal cellular repair), remain dormant until stimulated to divide (Bloom *et al.* 1966). The subjects chosen for this report had had chronic exposure to low levels of AGs as they worked in OTs which did not possess a scavenging system except for air-conditioners or fans.

Materials and methods

4 ml of blood per subject was collected in heparinised syringes, from 51 anaesthetists (31 men and 20 women), ranging in age from 28–60 years, who were in active service. Their exposure to anaesthetics ranged from 2 years to 29 years.

The controls chosen were matched with the subjects for sex, age (± 1 year), diet, smoking habits and lifestyle, from among the physicians who were not exposed to AGs. Smoker subjects

were matched with controls who smoked the same number of cigarettes per day.

Both subjects and controls were healthy, were not on antibiotics and provided medical and obstetric histories.

The cultures were set up according to the technique of Arakaki and Sparkes (1963) with minor modifications. The samples were set up for 48 hr. BrdU was added in the dark prior to incubation, to facilitate differentiation of M1 cycles from subsequent cell cycles. All procedures were made under sterile conditions. After termination, they were dropped on clean, cold wet coded slides, air dried, aged and stained by the FPG staining method (Perry and Wolff 1974), for differential staining of sister-chromatids. 100 M1 metaphases (1st cycle metaphases) were scored for aberrations in each subject and control as per the scoring criteria given in ISCN 1985. The results obtained were subjected to statistical evaluation.

Results

Chromosomal aberrations

The metaphase spreads were scored for the following structural chromosomal aberrations: chromatid gaps and breaks, chromosome gaps and breaks, exchanges, dicentrics, double minutes, ring chromosomes *etc.* A significant difference was seen in the number of aberrations in subjects compared to controls (Table 1). All aberrations were significantly greater in anaesthetists than in controls. There were significantly more chromatid gaps (Chi square=12.93, df=1, $p<0.001$) (Fig. 1), chromatid breaks (Chi square=6.47, df=1, $p<0.01$) (Fig. 2), chromosome gaps (Chi square=3.24, df=1, $p<0.05$) (Fig. 3), dicentrics (Chi square=7.80, df=1, $p<0.01$) (Fig. 5), fragments and double minutes (Chi square=23.99, df=1, $p<0.001$). Other chromosomal aberrations seen were chromosome breaks (Fig. 4), ring chromosomes (Fig. 6) and endoreduplication (Fig. 7) which did not differ significantly from that seen in controls. Table 2 shows the distribution of the various kinds of chromosomal aberrations in subjects and controls.

Univariate procedures were used to examine the influence of different independent variables on the number of chromosomal aberrations. No significant relationship was seen for age ($r=0.11$, df=49), years of exposure ($r=0.06$, df=49), or exposure in hr/day ($r=-0.03$, df=49).

The sex of the subject seemed to affect the number of chromosomal aberrations with male subjects showing more chromosomal aberrations than female subjects (4.81 ± 3.04 vs. 3.50 ± 1.82 , $t=1.92$, df=49, $p=0.06$). The influence of smoking on chromosome aberrations also showed a trend towards significance (non-smokers $n=44$, 4.55 ± 2.65 , smokers $n=7$, 2.71 ± 2.56 , $t=1.71$, df=49, $p=0.094$).

Discussion

The adverse effects of AGs in anaesthetists (as evidenced by the significantly greater number of CAs when compared to controls) is believed to be due to the exposure of the anaesthetists to the accumulation of waste AGs in the OT. This accumulation is marked in theaters which do not pos-

Table 1. Showing aberrations in subjects and controls

	No. of aberrations (Mean $\pm$ SD)	Significance
Anaesthetists (n=51)	4.29 ± 2.69	
Controls (n=51)	1.55 ± 1.54	$t=6.16$, df=50, $p<0.001$

Figs. 1-6. Chromosomal aberrations seen in anaesthetists. 1. Chromatid gap (ctg), 2. chromatid break (ctb), 3. chromosome gap (csg), 4. chromosome break (csb), 5. dicentric (dic), 6. ring chromosome (r).

Table 2. Presence (+) or absence (-) of different CAs in the sample

Aberrations	ct gaps		ct breaks		cs gaps		cs breaks		Rings		Dicentric		Exchanges		Tetraploidy		Others (frag+min)		All aberrations	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
51 Anaesthetists	36	15	15	36	34	17	12	39	3	48	9	42	4	47	9	42	21	30	46	5
51 Controls	17	34	4	47	24	27	6	45	0	51	0	51	3	48	3	48	0	51	35	16

sess a scavenging system, exposing the anaesthetists, surgeons, nurses, technicians and helpers to its possibly deleterious effects. Exposure concentrations are extremely variable, but may reach levels of at least 50 ppm for halothane and 5,000 ppm for nitrous oxide in unscavenged OTs (Baden and Simmons 1980).

The narcotic action of anaesthetics is due to its solubility in lipid cell membranes. The greater the solubility, the longer is its retention in the body, which in turn increases the time taken for elimination as well as its availability for biodegradation. This results in the accumulation of toxic and potentially mutagenic metabolites (Baden and Simmons 1980). Holmberg *et al.* (1982) suggests that AGs may act as a weak co-mutagen, which potentiates the genotoxic effects of other environmental factors, of which stress may be a prime candidate among anaesthetists. McNamee *et al.*

(1987) suggested that stress, fatigue and exposure to AGs may be responsible for the higher numbers of ill-health retirements and/or deaths among anaesthetists.

Volatile anaesthetics may be exhaled/excreted by the lungs, but are also metabolized *in vivo* by the cytochrome P-45 (mixed function oxidase) system, the enzymes of which are located in the S-ER of the liver and kidney. Halothane gets metabolized oxidatively to trifluoroacetic acid, bromine and chlorine. Trichloroacetaldehyde, an intermediary product is highly reactive and binds to tissue macromolecules. Bromochlorodifluoroethylene (BCD), another intermediary is a potent alkylating agent (Baden and Simmons 1980).

Nurse anaesthetists and female dental assistants, but not their male counterparts in the USA, were reported to show an increased incidence of cancer (Cohen *et al.* 1971). Animal experiments showed variable results when exposed to anaesthetics by oral lavage or inhalation (Basford and Fink 1968, Coate *et al.* 1979, Harper *et al.* 1982). Reports of foetal wastage among anaesthetists have been consistent (Vessey 1978, Corbett *et al.* 1974, Cohen *et al.* 1971). The incidence of spontaneous abortions (Cohen *et al.* 1971, Knill-Jones *et al.* 1972, 1975), congenital malformation and infertility have also been reported among female anaesthetists and wives of male anaesthetists.

At the cellular level, anaesthetics inhibit the mitotic spindle and produce c-metaphases (Sturrock and Nunn 1975, 1976), and non-disjunction in *Drosophila* (Clements and Todd 1981). Halothane showed weak mutagenic activity in long term treatments of *Drosophila*, showing a slight increase in mutation frequency (Kramers and Burn 1979, Kundomal and Baden 1985).

Cytogenetic reports on exposure to anaesthetics are few. Lamberti *et al.* 1989, Natarajan and Santhiya 1990, Karelova *et al.* 1992 and Padmavathi *et al.* 1995, consistently reported a significant increase in CAs in anaesthetists, and so is the finding in this study.

The significance of the present work lies in the fact that the subject sample was composed entirely of anaesthetists, the exposure time varying from 2 years to 29 years. This is important as anaesthetists are stationed close to the apparatus and therefore have the highest exposure (Gardner 1989). In the work of previous workers, even though the subject sample (anaesthetists, nurses and technicians) had variable exposures to the AGs (Table 3), there was a higher level of CAs in subjects (Lamberti *et al.* 1981, Natarajan and Santhiya 1992, Karelova *et al.* 1992, Padmavathy *et al.* 1995). Holmberg *et al.* (1982), however had only one anaesthetist in his sample, which may account

Table 3. Showing CA values

Author, year, sample chosen	CA values	Conclusion
Holmberg <i>et al.</i> (1982)		
11 subjects, (1)*	2.2	NS
10 controls	1.2	
Lamberti <i>et al.</i> (1989)		
30 subjects, (not specified)*	3.07	Significantly higher
15 controls	2.06	
Natarajan and Santhiya (1992)		
24 subjects, (11)*	mean values not provided	Significantly higher
24 controls		
Karelova <i>et al.</i> (1992)		
24 subjects, (14)*	3.84	Significantly higher
30 controls	1.87	
Padmavathi <i>et al.</i> (1995)		
36 subjects, (20)*	2.46	Significantly higher
20 controls	1.60	
Present work (1996)		
51 subjects, (51)*	4.29 ± 2.69	Significantly higher
51 controls	1.55 ± 1.54	

* The numbers in parentheses ( ) indicate the number of anaesthetists in the sample.

for his conclusion that CAs are not significantly higher in exposed personnel.

Another significant feature is that each subject was matched 1 : 1 with controls of the same sex, age (± 1 year), diet, smoking habits (even to the number of cigarettes smoked/day), and general life-style (practicing physicians). This was aimed at reducing the known confounding variables.

Chromosomal aberrations such as gaps, breaks, deletions, inversions and other structural rearrangements are the consequence of breaks in the DNA due to a mutagenic agent such as ionizing radiation or a chemical agent (anesthetics in this case). Chemical mutagens react with DNA, RNA and protein to form covalent adducts and cross-linking, which are primary events in mutagenesis and carcinogenesis (Sankaranarayanan 1994). Dimers formed between adjacent pyrimidines are the lesions that are ultimately translated into structural aberrations. These occur at specific sites, often referred to as 'hot-spots', usually located in the G-light chromosome bands (Chandley 1991).

Single-strand (SS) breaks are repaired normally, while base damages are repaired slowly (Natarajan 1984). The various enzymes routinely present in the cell, normally a) repair the breaks by joining the broken ends (restitution or healing) or b) there may be misrepair if there are several broken ends resulting in inversions and other structural rearrangements, or c) no repair at all resulting in gaps, breaks and deletions.

The effect of the mutagen on the chromosome depends on the phase of the cell at the time of exposure. If the exposure is just prior to the S-phase, (late G_1) or in the G_2 phase, the lesions are of the chromatid-type, confirming the split nature of the chromosomes after synthesis. Early G_1 insults result in the chromosome-type aberrations.

Chromosome aberrations were evaluated after 48 hr of culturing, as it has been reported (Evans and O'Riordan 1975) that most cells have completed the first mitotic division by this time. Analysis after 72 hr may show less CAs as repair processes may have taken place in the subsequent cell divisions. A decrease in breaks is associated with time due to repair mechanisms (Bryant and Slijepcevic 1993). The additional procedure of using the FPG stain helps to differentiate cells which have undergone more than 1 mitotic cycle even at 48 hr. Human peripheral lymphocytes are therefore particularly 'felicitous' for research purposes, as they can store the effects of damage until they are stimulated to undergo mitosis in culture (Wolff 1984).

The results obtained in the present work showed a significant difference of CAs in exposed subjects and unexposed controls. There was a marked increase of chromosomal aberrations like chromatid gaps and breaks, chromosome gaps, dicentrics, fragments and double minutes. Gaps have been included in this analysis, for even though gaps may not indicate true discontinuity, these are areas of despiralization which appear less well-stained (Therman 1986). Despiralization however represents a lesion, a structural modification, with loss of histone H1 protein and/or DNA, and this may be a response to a genotoxic insult. In this context, gaps assume significance.

The presence of chromatid breaks indicate that the damage was inflicted during the G_2 phase of the cell cycle and is consistent with reports of chemically induced damages (Therman 1986). Since circulating lymphocytes are in the G_0 phase of its life cycle, it is assumed that the effect of the mutagen (AGs), produces aberrations during the S phase, probably due to misreplication. *In vivo* exposure to mutagens produces DNA lesions in lymphocytes which are repaired by cellular repair enzymes. Unrepaired lesions give rise to the chromatid type of aberrations (Natarajan 1993). Unrepaired lesions also indicate that the cells are inherently repair-deficient—a pre-requisite in the production of cancer (Jaiswal *et al.* 1994). Almost all tumors in their advanced stages show chromosomal changes—either gain or loss (monosomy or trisomy) partial gain or loss (duplication, amplification, or deletion) or rearrangements (translocations and inversions), which alter the number, positional relationships, regulation (activation) or the integrity of genes. These changes may activate oncogenes as shown by familial colorectal cancer (Review by Cohen *et al.* 1993). It is therefore important not to ignore the significance of an increase in chromosomal aberrations in the anaesthetists as a consequence of exposure.

Conclusion and recommendation

Cytogenetic evaluation of 51 anaesthetists, presently working in Bangalore showed a significantly higher value of chromosomal aberrations like gaps, breaks, dicentric, fragments and double minutes than that seen in matched controls. This suggests that the chronic exposure by anaesthetists to low levels of anaesthetic gases that are ambient in the operation theater is capable of producing chromosomal aberrations in the peripheral lymphocytes of anaesthetists, which may predispose to cancer. It is suggested that there is a strong case for the urgent introduction of effective scavenging devices to reduce the level of ambient gas in the theater, and to reduce the occupational hazard that the gas poses to the health of all the personnel in the operation room.

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