SISTER CHROMATID EXCHANGES IN HUMAN LYMPHOCYTES
AFTER ANAESTHESIA WITH FLUROXENE

B. HUSUM, H. C. WULF AND E. NIEBUHR

SUMMARY

The potential mutagenicity of fluroxene was investigated by the sister chromatid exchange (SCE) test using
lymphocytes in peripheral blood from patients before and after anaesthesia. Twenty-five female patients,
aged 21–77 yr (median 36.5 yr) were anaesthetized for gynaecological operations with fluroxene in nitrous
oxide for 60–220 min (median 110 min). The number of SCE per cell was the same before and immediately
after anaesthesia. In 10 patients, SCE were examined 3 days later and no increase was observed. In nine of the
other patients, SCE rates were identical before and after anaesthesia and 1 and 5 days later. It was concluded
that there was no indication, from this test, of a mutagenic effect of short-term exposure to anaesthetic
concentrations of fluroxene in nitrous oxide.

The possibility of a health hazard from anaesthetic
agents has been discussed extensively for the last
decade (reviews by Spence and Knill-Jones, 1978;
Cohen, 1980; Vessey and Nunn, 1980). Experimental
and epidemiological studies have indicated indirectly that working in an environment contaminated
by waste anaesthetic gases may be a health hazard,
but a casual relationship has never been established.

Recently, laboratory tests on mutagenicity have
been used in investigations of inhalation anaesthetics (Baden et al., 1977; White et al., 1979; Baden
and Simmon, 1980; Basler and Röhborn, 1981). The
sister chromatid exchange test is based on
examination of the exchange of DNA material be-
tween the two chromatids in the chromosomes in
mammalian cells. An increased number of such
sister chromatid exchanges (SCE) reflects the influ-
ence of mutagens (Perry and Evans, 1975; Latt et
al., 1979). Previous in vitro studies of inhalation
anaesthetics by this test showed only the vinyl-
containing compounds fluroxene, ethyl-vinyl ether
and divinyl ether increased SCE, suggesting that the
vinyl moiety might be important in this capacity
(White et al., 1979).

The SCE test may also be useful for evaluation of
exposure to potential mutagens in vivo (Latt et al.,
1979; Wulf, 1980). Application of this test using
lymphocytes in peripheral blood from anaesthetized
humans has revealed no indication of a mutagenic
effect of short-term exposure to anaesthetic concen-
trations of halothane or enflurane in nitrous oxide
(Husum, Wilf and Niebuhr, 1981a).

In the present study, we examined SCE in lym-
phocytes in peripheral blood drawn from patients
before and after anaesthesia with fluroxene in nit-
rous oxide.

MATERIAL AND METHODS

The study was performed in 25 female patients,
aged 21–77 yr (median 36.5 yr) who underwent op-
eration for non-malignant gynaecological disease.
All patients were otherwise healthy and had not
received regular medication before operation. In-
formed consent was obtained from all patients at the
preoperative visit.

A venous heparinized blood sample was taken and
then anaesthesia induced with thiopentone and
maintained with 3–5% fluroxene in nitrous oxide in
oxygen (2:1). A second venous blood sample was
taken from all patients after completion of anaes-
thesia. In 10 patients, a third blood sample was
obtained 3 days after the operation and in nine other
patients, blood samples were taken on the 1st and
5th days after operation.

The heparinized venous blood samples were
labelled with code numbers so that investigators
were unaware of the time of withdrawal. A 0.5-ml
aliquot of blood from each sample was incubated in
9 ml of Parker 199 standard medium with 15% fetal
calf serum, phytohemagglutinin (PHA) 0.2 ml and
5-bromo-2'-deoxyuridine (BrdU) 2 × 10⁻⁵
mol litre⁻¹ (6 μg ml⁻¹). The cells were grown at 37°C
for 72 h and cell division stopped during the last 2 h

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by adding Colcemid $3 \times 10^{-7}$ mol litre$^{-1}$ to the medium. The cells were treated with hypotonic potassium chloride 75 mmol litre$^{-1}$ and fixed with glacial acetic acid in methanol (1:3). Treatment with bisbenzimide and ultraviolet light thereafter made old and newly synthesized DNA material colour differently when stained with Giemsa (Perry and Wolff, 1974; Wulf, 1980). Thirty metaphases were scored for SCE in each specimen, one SCE being counted each time two adjacent segments of a chromatid were differently coloured.

**Statistical methods** (Husum, Wulf and Niebuhr 1981b): the number of SCE in different cells of an individual person was assumed to behave as coming from a mixture of Poisson distributions. The sum of SCE in 30 cells from each patient therefore followed a Poisson distribution, and the transformation

$$ y = \sqrt{\sum \text{SCE}} + \sqrt{\sum \text{SCE} + 1} $$

produced a normally distributed variable $y$ by which a possible effect of anaesthesia with fluroxene might be evaluated. A $t$ test was used to compare the mean values of the variable $y$ in all patients before and after anaesthesia. The mean values of $y$ at 1, 3 and 5 days after operation were compared with the corresponding values before and after anaesthesia by analysis of variance. Differences were considered to be statistically significant when $P$ was less than 0.05.

**RESULTS**

Before induction of anaesthesia, the 25 patients had $9.91 \pm 0.39$ SCE per cell (mean $\pm$ SEM). Immediately after anaesthesia the values were $9.98 \pm 0.39$ SCE per cell (mean $\pm$ SEM) (table I). Ten patients had identical SCE rates before, immediately after, and 3 days after anaesthesia (table II), and nine other patients had identical SCE rates before and after anaesthesia and 1 and 5 days later (table III).

**TABLE I.** Sister chromatid exchanges (SCE) in lymphocytes in 25 female patients, aged 21–77 yr (median 36.5 yr), who received fluroxene in nitrous oxide in oxygen (2:1) for 60–220 min (median 110 min)

<table>
<thead>
<tr>
<th>SCE per cell</th>
<th>$y = \sqrt{\sum \text{SCE}} + \sqrt{\sum \text{SCE} + 1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean $\pm$ SEM</td>
</tr>
<tr>
<td>Before anaesthesia</td>
<td>9.91 $\pm$ 0.39</td>
</tr>
<tr>
<td>After anaesthesia</td>
<td>9.98 $\pm$ 0.39</td>
</tr>
</tbody>
</table>

**TABLE II.** Sister chromatid exchanges (SCE) in lymphocytes in 10 female patients before and after anaesthesia with fluroxene in nitrous oxide. Using analysis of variance, no statistically significant difference was observed ($F = 0.0361$ with 2 and 27 degrees of freedom)

<table>
<thead>
<tr>
<th>SCE per cell</th>
<th>$y = \sqrt{\sum \text{SCE}} + \sqrt{\sum \text{SCE} + 1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean $\pm$ SEM</td>
</tr>
<tr>
<td>Before anaesthesia</td>
<td>10.36 $\pm$ 0.87</td>
</tr>
<tr>
<td>After anaesthesia</td>
<td>10.43 $\pm$ 0.81</td>
</tr>
<tr>
<td>3rd day after op.</td>
<td>10.15 $\pm$ 0.62</td>
</tr>
</tbody>
</table>

**TABLE III.** Sister chromatid exchanges (SCE) in lymphocytes in nine female patients before and after anaesthesia with fluroxene in nitrous oxide. Using analysis of variance, no statistically significant difference was observed ($F = 0.1867$ with 3 and 32 degrees of freedom)

<table>
<thead>
<tr>
<th>SCE per cell</th>
<th>$y = \sqrt{\sum \text{SCE}} + \sqrt{\sum \text{SCE} + 1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean $\pm$ SEM</td>
</tr>
<tr>
<td>Before anaesthesia</td>
<td>9.84 $\pm$ 0.37</td>
</tr>
<tr>
<td>After anaesthesia</td>
<td>9.60 $\pm$ 0.47</td>
</tr>
<tr>
<td>1st day after op.</td>
<td>10.09 $\pm$ 0.41</td>
</tr>
<tr>
<td>5th day after op.</td>
<td>10.01 $\pm$ 0.61</td>
</tr>
</tbody>
</table>
SCE AFTER FLUROXENE ANAESTHESIA

DISCUSSION

Using the Ames test (Ames, McCann and Yamasaki, 1975), Baden and colleagues (1977) found a positive mutagenicity for fluroxene but negative results for halothane and enflurane. A later study (Baden et al., 1978) confirmed that fluroxene was mutagenic to Salmonella typhimurium in the presence of a rat liver enzyme system, but not in the presence of human liver preparations. White and others (1979) examined SCE in Chinese hamster ovary (CHO) cells suspended in a medium containing a rat liver enzyme system. Following a 1-h exposure in vitro to 1 MAC of 10 inhalation anaesthetics, only the vinyl-containing compounds increased SCE. When the CHO cells were exposed to 1 MAC of each of the various agents for 24 h without liver microsomal enzymes being present, no increase in SCE was observed and 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. However, in man unlike other species, fluroxene is primarily biotransformed to trifluoroacetic acid which is believed to be without serious toxic effects (Johnston et al., 1973).

In a thorough study of exposure in vivo, Basler and Röhrborn (1981) exposed Chinese hamsters and mice to various clinical concentrations of halothane and examined bone marrow cells for structural chromosome aberrations, micronuclei, SCE and dominant lethal mutations. They found no evidence of a mutagenic effect of halothane.

Application of the SCE test on human cells exposed in vitro has not revealed any change in SCE following exposure to halothane or enflurane in anaesthetic concentrations (Husum, Wulf and Niebuhr, 1981a). In the present study, we examined SCE in lymphocytes from peripheral blood in patients exposed to anaesthetic concentrations of fluroxene in nitrous oxide. Application of this method, comprising human cells exposed in vivo, appeared to be justified because it allowed testing of the biotransformation products of fluroxene and of the compound itself.

The study revealed no change in SCE following short-term exposure to fluroxene in anaesthetic concentrations and agrees with results of previous laboratory investigations in non-human cells exposed in vitro in which mutagenicity of fluroxene was demonstrated only in the presence of enzymes prepared from the livers of Aroclor 1254-pretreated rodents (Baden et al., 1978; White et al., 1979).

REFERENCES


Chez neuf autres patientes, les fréquences d'ECS étaient les mêmes avant et après l'anesthésie et 1–5 jours plus tard. Nous en concluons que d’après ce test, il n’y a pas d’indices d’un effet mutagène d’une exposition brève à des concentrations anesthésiques de fluoroxyne dans le protoxyde d’azote.

AUSTAUSCH DES SCHWESTER-CHROMATIDS IN MENSCHLICHEN LYMPHOZYTEN NACH NARKOSE MIT FLUROXEN

ZUSAMMENFASSUNG

INTERCAMBIOS CROMATIDOS GEMELOS EN LINFOCITOS HUMANOS DESPUÉS DE LA ANESTESIA CON FLUROXENO

SUMARIO
Se investigó el aspecto mutagénico potencial del fluroxeno mediante la prueba de intercambio cromático gemelo, haciendo uso de linfocitos del riego sanguíneo periférico de los pacientes antes y después de la anestesia. Se anestesió a 25 pacientes femeninos, de edades comprendidas entre 21 y 77 años (media de 36,5 años) para someterlas a operaciones ginecológicas, utilizando para ello fluroxeno en óxido nitroso por espacio de 60 a 220 minutos (media de 110 minutos). El número de intercambios cromáticos gemelos por célula fue idéntico antes y después de la anestesia. Se examinó el cambio cromático gemelo en 10 pacientes al cabo de tres días sin observarse incremento alguno. En otros 9 pacientes el régimen de intercambio fue idéntico antes y después de la anestesia después de transcurridos 1 y 5 días. Se concluyó que esta prueba no aportó indicación alguna de un efecto mutagénico como consecuencia de la exposición a corto plazo a concentraciones anestésicas de fluoroxyne en óxido nitroso.