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 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 Röhrborn, 1981). The sister chromatid exchange test is based on examination of the exchange of DNA material between the two chromatids in the chromosomes in mammalian cells. An increased number of such sister chromatid exchanges (SCE) reflects the influence 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 concentrations of halothane or enflurane in nitrous oxide (Husum, Wilf and Niebuhr, 1981a).

In the present study, we examined SCE in lymphocytes in peripheral blood drawn from patients before and after anaesthesia with fluroxene in nitrous oxide.

MATERIAL AND METHODS

The study was performed in 25 female patients, aged 21–77 yr (median 36.5 yr) who underwent operation for non-malignant gynaecological disease. All patients were otherwise healthy and had not received regular medication before operation. Informed 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 anaesthesia. 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) $2 \times 10^{-5}$ 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.
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

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

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 ± SEM). Immediately after anaesthesia the values were $9.98 \pm 0.39$ SCE per cell (mean ± 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>$y = \sqrt{\text{sum SCE}} + \sqrt{\text{sum SCE} + 1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Before anaesthesia</td>
<td>9.91</td>
</tr>
<tr>
<td>After anaesthesia</td>
<td>9.98</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>$y = \sqrt{\text{sum SCE}} + \sqrt{\text{sum SCE} + 1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Before anaesthesia</td>
<td>10.36</td>
</tr>
<tr>
<td>After anaesthesia</td>
<td>10.43</td>
</tr>
<tr>
<td>3rd day after op.</td>
<td>10.15</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>$y = \sqrt{\text{sum SCE}} + \sqrt{\text{sum SCE} + 1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Before anaesthesia</td>
<td>9.84</td>
</tr>
<tr>
<td>After anaesthesia</td>
<td>9.60</td>
</tr>
<tr>
<td>1st day after op.</td>
<td>10.09</td>
</tr>
<tr>
<td>5th day after op.</td>
<td>10.01</td>
</tr>
</tbody>
</table>
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 vitro, 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).

**DISCUSSION**

**REFERENCES**


**ECHANGES DE CHROMATIDES SOEURS DANS DES LYMPHOCyTES HUMAINS APRES UNE ANESTHESIE AU FLUROXENE**

**RESUME**

La mutagénicité potentielle du fluroxène a été étudiée par le test d’échange des chromatides sour (ECS) en utilisant des lymphocytes du sang périphérique de patients avant et après l’anesthésie. Vingt cinq femmes, âgées de 21 à 77 ans (moyenne: 36,5 ans) ont été anesthésiées pour des interventions gynécologiques au fluroxène dans le protoxyde d’azote durant 60 à 220 min (moyenne: 110 min). Le nombre d’ECS par cellule était le même avant et juste après l’anesthésie. Chez dix patientes, les ECS ont été examinés 3 jours plus tard, et aucune augmentation n’a été notée.

**ACKNOWLEDGEMENT**

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**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 fluoroène 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 fluorozeno 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 fluorozeno 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 paciente 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 fluorozeno en óxido nitroso.