SISTER CHROMATID EXCHANGES IN CIGARETTE SMOKERS: EFFECTS OF HALOTHANE, ISOFLURANE OR SUBARACHNOID BLOCKADE

B. HUSUM, N. VALENTIN, H. C. WULF, A. HALABURT
AND E. NIEBUHR

Testing of the potential mutagenic effects of inhalation anaesthetics by the sister chromatid exchange (SCE) test in lymphocytes from patients undergoing surgery has yielded negative responses following anaesthesia with halothane (Husum, Wulf and Niebuhr, 1981a; Holmberg et al., 1982), enflurane (Husum, Wulf and Niebuhr, 1981a) and fluoroxene (Husum, Wulf and Niebuhr, 1982a). In a similar study of isoflurane (Husum et al., 1984) there was a negative response in non-smoking patients, whereas SCE increased significantly in a subset of 11 patients who were cigarette smokers—when examined on the day following the operation. It is known that cigarette smokers have increased SCE in lymphocytes in peripheral blood (Lambert et al., 1978; Husum, Wulf and Niebuhr, 1982b), but it is unknown whether cigarette smokers react differently from non-smokers when exposed to potential SCE-inducing agents (Watanabe et al., 1983).

In the present study, the possible SCE-inducing effect of anaesthetic agents was studied in patients who were cigarette smokers. In a total of 63 regular cigarette smokers, SCE was examined before and after surgery under general anaesthesia (with halothane or isoflurane) or in association with subarachnoid analgesia.

SUMMARY
In a previous study of the potential mutagenic action of isoflurane using the sister chromatid exchange (SCE) test in lymphocytes of surgical patients, it appeared that SCE increased in a group of 11 cigarette smokers, there being no effect in patients who were non-smokers. In the present study, 63 cigarette smokers were examined by the SCE test before and after minor orthopaedic operations undertaken under halothane or isoflurane anaesthesia, or subarachnoid analgesia. No significant changes of SCE were observed, and the risk of having missed a "true" increase of more than 0.6 SCE per cell was less than 1%. It was concluded that, in cigarette smokers, SCE in lymphocytes were unchanged after both general anaesthesia and subarachnoid analgesia, and that there was no indication from the SCE test of a mutagenic action of halothane, or isoflurane, in nitrous oxide.

PATIENTS AND METHODS
Sixty-three patients aged 18–64 yr (median 35.3 yr) who underwent minor orthopaedic surgery were studied. There were 23 women aged 18–64 yr (median 36.0 yr), and 40 men aged 19–63 yr (median 35.0 yr). All patients were healthy and had received no regular medication before operation. All were regular cigarette smokers, their average daily cigarette consumption being 15.2 cigarettes (range 10–30 cigarettes per day) (table I).

The investigation was approved by the Copenhagen County Ethical Committee. Informed

B. HUSUM, M.D., Department of Anaesthesia, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark. N. VALENTIN, M.D.; A. HALABURT, M.D.; Department of Anaesthesia, Gentofte Hospital, Copenhagen, H. C. WULF, M.D., PH.D., Department of Dermatology, The Finsen Institute, Copenhagen. E. NIEBUHR, M.D., PH.D., The University Institute of Medical Genetics, Copenhagen.

Correspondence to B. H.
TABLE I. Demographic data for 63 patients in whom sister chromatid exchanges (SCE) were examined before anaesthesia and on the day after the operation

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. patients</th>
<th>Age (yr)</th>
<th>Cigarettes per day</th>
<th>Duration of anaesthesia (min)</th>
<th>Cigarette consumption between 1st and 2nd sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median Range</td>
<td>Median Range</td>
<td>Median Range</td>
<td>Median Range</td>
</tr>
<tr>
<td>Halothane</td>
<td>20</td>
<td>34.5 20-64</td>
<td>16.5 10-30</td>
<td>63 35-115</td>
<td>4.0 0-25</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>23</td>
<td>35.5 18-63</td>
<td>17.5 10-30</td>
<td>80 30-120</td>
<td>2.8 0-8</td>
</tr>
<tr>
<td>Subarachnoid analgesia</td>
<td>20</td>
<td>38.5 19-57</td>
<td>13.7 10-20</td>
<td>4.0 0-8</td>
<td></td>
</tr>
</tbody>
</table>

Consent was obtained from all patients at the preoperative visit. The patients were asked to refrain from smoking from the midnight before the operation and for the following 36 h. In all patients premedication consisted of pethidine 1 mg kg\(^{-1}\) i.m.

In two of the groups, anaesthesia was induced with either halothane or isoflurane with 67% nitrous oxide in oxygen. Tracheal intubation was necessary in a few patients, but was performed always without the use of myoneural blockers. Pethidine was used to supplement anaesthesia and to provide pain relief in the period after surgery. In a third group, analgesia was produced by the intrathecal injection of 0.5% bupivacaine 4-5 ml.

Venous blood was sampled before the induction of anaesthesia and again on the day after operation. Blood samples were labelled with code numbers so that the investigators were unaware of the time of withdrawal.

The SCE analyses were undertaken using the method described by Wulf (1980). 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.** Using the approach described previously (Husum, Wulf and Niebuhr, 1981b; Wulf et al., 1984), the sum of SCE in 30 cells from each patient was transformed into a normally distributed variable \(y = (\text{sum SCE})^1 + (\text{sum SCE} + 1)^1\). A two-tailed paired \(t\) test was used to compare the mean values of the variable \(y\) in each of the groups of patients before and after the operation. Analysis of variance was used to compare the observations in the three groups.

**RESULTS**

Before the induction of anaesthesia, the 63 patients had 9.15 ± 0.21 SCE per cell (mean ± SEM), and there was no significant difference between the mean SCE values in the three treatment groups (table II). On the day after the operation, the 63 patients averaged 8.94 ± 0.19 SCE per cell; that is,
SCE had not changed significantly from the corresponding values observed before the induction of anaesthesia in any of the three treatment groups (table II).

DISCUSSION

The possibility of a potential mutagenic action of inhalation anaesthetics has been investigated with the Ames Salmonella assay system (Baden et al., 1977; Waskell, 1978; Baden and Simmon, 1980) and with the SCE test following exposure in vitro (White et al., 1979) and in vivo (Basler and Röhrborn, 1981; Husum, Wulf and Niebuhr, 1981a, 1982a; Holmberg et al., 1982; Husum et al., 1984).

Increased SCE in lymphocytes has been demonstrated as early as 75 min after the administration of a known SCE-inducing agent (Wulf et al., 1984) and an increased value of SCE following acute exposure is believed to persist for at least 4–16 weeks (Vainio et al., 1981). Therefore, it was reasonable to assume that the design of the present study would allow the detection of any change in SCE which might have been induced during the period of anaesthesia and surgery.

The three study groups were comparable with respect to cigarette consumption and pertinent personal data (table I), and before the operation there were no differences in SCE between the groups (table II). The number of SCE per cell was the same as observed in a previous study of SCE in healthy cigarette smokers (Husum, Wulf and Niebuhr, 1982b).

The duration of anaesthesia was in the same range in both groups receiving general anaesthesia. In the patients receiving subarachnoid analgesia, injection of 0.5% bupivacaine 4–5 ml typically produced sensory blockade up to the lower thoracic segments.

On the day after the operation, there were no significant changes in SCE in any of the three groups (table II), and there were no differences in postoperative SCE values between the three treatment groups. From estimates of the 99% confidence limits from the mean differences between SCE per cell before and after the operation, it appeared that the risk of having missed a "true" increase of more than 0.6 SCE per cell was 1% or less in all the three groups.

This is in contrast to our earlier study on patients anaesthetized with isoflurane (Husum et al., 1984), in which 11 patients who were cigarette smokers (average 10 cigarettes per day) averaged 8.57 ± 0.38 per cell before the operation and 9.38 ± 0.36 SCE per cell on the day after the operation. This increase of 0.81 SCE per cell was significant at the 2% level. The patients were undergoing the same surgical procedures and the anaesthetic procedures were quite similar in the two studies, except that diazepam was used for premedication in the earlier study. We do not believe that the oral intake of a single, large dose of diazepam is a confounding factor since, according to a recent study, SCE is not influenced by such medication (Husum et al., 1985).

We conclude that, in cigarette smokers undergoing minor orthopaedic surgery, SCE in lymphocytes were unchanged after both general anaesthesia and subarachnoid analgesia, and that there was no indication, from the SCE test, of a mutagenic action as a result of anaesthesia with isoflurane, or halothane, in nitrous oxide.

ACKNOWLEDGEMENTS

Technical help in the laboratory was given by Lizzie Hansen and Lone Studstrup, and secretarial help was given by Susanne Dahlberg and Birgitte Neergaard.

REFERENCES


of patients treated with single, large doses of diazepam. 


