NO MUTAGENIC EFFECT OF ENFLURANE ON CULTURED CELLS

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SUMMARY
Enflurane was tested by the 8-azaguanine system for any evidence of mutagenic effects. No significant increase in the number of mutant colonies compared with controls was found after 24 h of exposure of Chinese hamster cells to 1.5–6.5% enflurane and no damaged chromosomes were seen in metaphase cells on slides. Studies of inhibition of growth rate by enflurane show that in this respect its effects were similar in magnitude to other commonly used inhalation anaesthetic agents, but with regard to cell survival (as measured by colony-forming ability) there was surprisingly little increase in toxicity with increasing concentrations of enflurane.

Some recent papers have drawn attention to the value of mutagenicity testing as a screen for carcinogenicity. Corbett (1976) has described a series of experiments which suggested the possibility that the anaesthetic agent isoflurane might be a transplacental carcinogen. Also, there are epidemiological reports of the increased frequency of some malignancies noted by various workers (Corbett et al., 1973; American Society of Anesthesiologists, 1974).

Classical carcinogenicity testing in vivo requires the administration of high doses of agents, for large fractions of the life span, to several species of laboratory animals and, in the case of inhalation anaesthetics, such experiments are difficult technically. Meanwhile, mutagenesis testing in vitro provides a rapid screening assay.

McCann and others (1975) and McCann and Ames (1976) tested 175 known carcinogens and found 90% of these chemical substances to be mutagenic. Of the 18 carcinogens that were not mutagenic in the Salmonella/microsome test, nine were found later to be mutagenic in other systems (de Serres, 1976).

A previous paper (Sturrock, 1977) reported negative mutagenesis results for halothane, chloroform and nitrous oxide, using the cultured fibroblast/8-azaguanine system and this work has now been extended to include enflurane.

METHODS
Chinese hamster (CH) lung fibroblast cells, line V.79, were grown in bottles, as described previously (Sturrock, 1977) and exposed to enflurane. Enflurane, in a carrier gas of air with 5% carbon dioxide, was vaporized in an Enfluratec (Cyprane Ltd, Keighley, Yorks) and delivered at a flow rate of 300 ml min⁻¹ for 1 h. The anaesthetic concentration in the gas phase was measured with a Riken refractometer. After 1 h the bottles were sealed and stored for a further 23 h in a hot room at 37 °C. Subsequently, the cells were collected by trypsinization, counted, diluted and seeded into two sets of Petri dishes for determining cell survival, as shown by colony-forming ability (CFA) and production of mutant colonies, as described previously (Sturrock, 1977). Other cells growing on glass slides in Petri dishes within desiccators were exposed to enflurane also.

Control bottles were gassed with 5% carbon dioxide in air and their contents treated exactly as the test cells.

RESULTS
Results were calculated as before (Sturrock, 1977). Table I shows growth rate, CFA and numbers of mutant colonies found after exposure of CH cells to 1.5%, 2.5%, 4.5% or 6.5% enflurane for 24 h, together with figures for the control cells. Man-MAC for enflurane is 1.68% (Gion and Saidman, 1971).

Figure 1 shows the effect of six different volatile anaesthetic agents on growth rate (A) and on colony-forming ability (B) in CH cells. For the sake of clarity the points have been omitted from all the lines except those for enflurane. Growth rate was reduced considerably by exposure to enflurane but CFA, which measures long-term viability of cells, was affected much less. Mutation frequency in treated cultures was actually lower than for the control cells, except for one result, which was not significantly different from the control. No breaks or other damage were seen in the chromosomes of cells on slides that had been exposed to enflurane.
Table I. Growth rate, colony-forming ability and mutation frequency in CH cells after exposure to enflurane for 24 h

<table>
<thead>
<tr>
<th></th>
<th>Enflurane (%)</th>
<th>Growth rate (% control)</th>
<th>CFA (% control ± 2 SEM)</th>
<th>Mean mutant colonies per dish</th>
<th>Mutation frequency per 10^8 surviving cells</th>
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</thead>
<tbody>
<tr>
<td>Series I</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>68</td>
<td>84 ± 1.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>47</td>
<td>72 ± 1.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>42</td>
<td>68 ± 1.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>30</td>
<td>61 ± 2.2</td>
<td>—</td>
<td>—</td>
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<tr>
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<td>100</td>
<td>0.6</td>
<td>16</td>
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<tr>
<td></td>
<td>1.5</td>
<td>71</td>
<td>86 ± 2.9</td>
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<tr>
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<td>2.5</td>
<td>42</td>
<td>80 ± 3.7</td>
<td>0.3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>40</td>
<td>75 ± 1.3</td>
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<td>13</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>25</td>
<td>71 ± 1.8</td>
<td>0.5</td>
<td>19</td>
</tr>
</tbody>
</table>

DISCUSSION

It is clear that exposure to enflurane for 24 h is not mutagenic to CH cells, as determined by the 8-azaguanine system. Baden, Brinkenhoff and others (1976) reported negative results for mutagenesis by halothane, using the Salmonella/microsome system and, more recently, Baden, Kelly and others (1976) have given preliminary notice of further negative studies including enflurane, isoflurane and methoxyflurane. Only fluroxene was found to be mutagenic in their system. Therefore it seems most unlikely that enflurane, which has proved to be non-mutagenic in two different test systems, could have any carcinogenic potential.

The study has also provided information on the effects of enflurane on growth rate and colony-forming ability in CH cells in relation to some other volatile anaesthetic agents, and, in figure 1, it can be seen that the magnitude of the effect on growth rate is roughly proportional to anaesthetic potency. However, the slope of the regression line for the inhibition of CFA by enflurane (fig. 1B) is remarkably shallow, indicating that this anaesthetic has a low toxic effect on CH cell survival.

ACKNOWLEDGEMENT

Enflurane was supplied by Abbott Laboratories Limited.

REFERENCES


NO MUTAGENIC EFFECT OF ENFLURANE ON CULTURED CELLS


KEINE MUTAGENE WIRKUNG VON ENFLURAN AUF ZELLKULTUREN

ZUSAMMENFASSUNG

Mittels des 8-Azaguanin-Systems wurde Enfluran auf Hinweise für mutagene Wirkungen getestet. Nach einer 24-stündigen Aussetzung der Zellen eines chinesischen Hamsters von 1,5–6,5% Enfluran wurde kein wesentlicher Anstieg in der Anzahl mutierter Zellkolonien im Vergleich mit den Kontrollzellen festgestellt, und in den Metaphase- zellen wurden inter dem Mikroskop keine beschädigten Chromosomen gefunden. Untersuchungen über die Behinderung der Wachstumsrate durch Enfluran zeigen, dass dabei die Wirkungen dieser Droge der Größenordnung nach ähnlich sind wie die anderer, normalerweise verwendeter Einatmungsnarkotika; was aber das Überleben von Zellen betrifft (gemessen an der Fähigkeit zur Kolonienbildung), so gab es bei steigenden Konzentrationen von Enfluran einen überraschend geringen Anstieg in der Toxizität.

CARENCIA DE EFECTO MUTAGENICO DEL ENFLURANO SOBRE CELULAS CULTIVADAS

SUMARIO

Se sometió a prueba el enfuran, por el método de la 8-azaguánina, para determinar cualquier evidencia de efectos mutagénicos. No se halló aumento significativo en la cifra de colonias mutantes, comparado con la de los testigos, tras 24 h de exposición de células de criceto asiático a 1,5–6,5% enfluran, y los portos con células en metafase no evidencieron cromosomas dañados. Los estudios de inhibición de la tasa de crecimiento por el enfluran muestran que a este respecto sus efectos son similares en magnitud a los de otros agentes anestésicos de inhalación empleados comúnmente, pero en cuanto a supervivencia celular (medida según la facultad de formar colonias) existe un aumento sorprendentemente pequeño de la toxicidad según aumentan las concentraciones de enfluran.