SEX DIFFERENCES IN ANAESTHETIC TOXICITY: FLUROXENE AND TRIFLUOROETHANOL IN MICE

H. F. CASCORBI, B. H. GORSKY AND J. E. REDFORD

SUMMARY

A sex difference in postanaesthetic mortality after fluroxene anaesthesia was found in Swiss Webster mice. More males succumbed than females. This toxicity was biotransformation-dependent and could be reversed by pretreatment with "opposite" sex hormones. The toxicity of the fluroxene metabolite trifluoroethanol also was more marked in male mice, but was only partially influenced by microsomal enzyme inhibitors or stimulators, or by sex hormones.

Fluroxene (Fluoromar) has been shown to cause delayed death in mice (Cascorbi and Singh-Amaranath, 1972). It has been demonstrated that if anaesthesia with approximately 4.5% fluroxene exceeds 1 h, the animals recover apparently unharmed. However, many die within the subsequent 30 h. The number of deaths after fluroxene anaesthesia increases with increasing duration of anaesthesia and may be altered by pretreatment with either enzyme-inducing or enzyme-suppressing drugs. Phenobarbitone dramatically increases the toxicity of fluroxene whereas a small dose of carbon tetrachloride abolishes it. It is likely that trifluoroethanol (TFE), one of the major metabolites of fluroxene in mice (Blake et al., 1967), is responsible for death following fluroxene anaesthesia (Blake et al., 1969).

The influence of sex hormones on the biotransformation of volatile anaesthetics has not been studied previously. Despite species differences, it appears that in some mouse strains the rate of hexobarbitone metabolism in females is slower than in males (Westfall et al., 1964). If this were true for fluroxene biotransformation, female mice should be more resistant to postanaesthetic toxicity than males. Since there is some evidence that it is a metabolite of TFE rather than TFE itself which is toxic (Blake et al., 1969; Cascorbi and Singh-Amaranath, 1973), a difference in toxicity of this compound in male and female mice should be demonstrable. The clinical importance of fluroxene is negligible. However, it deserves attention as a model in which to study the toxic mechanisms of halogenated anaesthetic agents.

METHODS

Male and female Swiss Webster mice (20–30 g body weight) (Carworth Farms, New York, N.Y.) were kept in groups of 10–15 in metal case cages on pinewood chips, fed Purine Mouse Chow and water ad libitum for at least 1 week. The cages were in an air-conditioned laboratory with a diurnal cycle, and care was taken to avoid exposure to enzyme-inducing xenobiotics except for the pinewood chips (Cascorbi and Singh-Amaranath, 1973).

Groups of animals were pretreated as follows:

1. No drugs.
2. Carbon tetrachloride, 20% in Wesson Oil, 0.2 ml s.c. per mouse, 24 h before anaesthesia.
3. Phenobarbital sodium USP, 25 mg%, in the drinking water for 5 days before anaesthesia.
4. Testosterone (Lilly) 5 mg s.c. per mouse, for 12 days in female mice.
5. Oestradiol benzoate (Schering) 100 µg s.c. per mouse for 12 days in male mice.

In addition, four litters of Swiss Webster mice, reared in our laboratory, were weaned and anaesthetized with fluroxene at approximately 4 weeks of age (5 g body weight).

Approximately 4% fluroxene was administered for 1 or 7 h. The animals were anaesthetized in desiccators using a high flow of air through a Fluoromartec. Similar groups received TFE 175 and 250 mg/kg (Baker Chemical) i.p. using an aqueous solution (3 vol%). In preliminary experiments this dose range was found to produce the desired toxicity.

After anaesthesia or TFE injection, the animals were observed for 48 h and the frequency of death was noted. Where necessary, the results were evaluated using the χ² test.
RESULTS

Fluroxene

Table I shows that after 1 h of fluroxene anaesthesia, about one-third of the males died, whereas none of the females succumbed. Seven hours of fluroxene anaesthesia was necessary to reveal the postanaesthetic toxicity of fluroxene in female mice.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Hours of anaesthesia</th>
<th>n</th>
<th>Sex</th>
<th>% dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
<td>44</td>
<td>M</td>
<td>37</td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>44</td>
<td>F</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>16</td>
<td>F</td>
<td>25</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>1</td>
<td>35</td>
<td>M</td>
<td>0</td>
</tr>
<tr>
<td>Testosterone</td>
<td>1</td>
<td>28</td>
<td>F</td>
<td>44</td>
</tr>
<tr>
<td>Phenobarbitone</td>
<td>1</td>
<td>18</td>
<td>M</td>
<td>100</td>
</tr>
<tr>
<td>Phenobarbitone</td>
<td>1</td>
<td>30</td>
<td>F</td>
<td>93</td>
</tr>
<tr>
<td>CCl₄</td>
<td>1</td>
<td>18</td>
<td>M</td>
<td>0</td>
</tr>
<tr>
<td>CCl₄</td>
<td>7</td>
<td>10</td>
<td>F</td>
<td>0</td>
</tr>
<tr>
<td>Immature</td>
<td>1</td>
<td>27</td>
<td>M</td>
<td>0</td>
</tr>
<tr>
<td>Immature</td>
<td>1</td>
<td>16</td>
<td>F</td>
<td>0</td>
</tr>
</tbody>
</table>

Pretreatment with phenobarbitone increased postanaesthetic fluroxene toxicity in both sexes: almost all of the females and all of the males succumbed following 1 h of fluroxene anaesthesia after phenobarbitone pretreatment. It is noteworthy that, in this experiment, the males died within a few hours of anaesthesia, whereas the females survived for considerably longer. Carbon tetrachloride pretreatment decreased the toxicity of fluroxene in both sexes. Treatment with the "opposite" sex hormone protected the males against fluroxene toxicity, and the females became as susceptible as the males. There was no demonstrable sex difference in postanaesthetic fluroxene toxicity after 1 h of anaesthesia in immature male or female mice.

Trifluoroethanol

Table II summarizes the results of TFE injections. Untreated mice showed a sex difference in the toxicity of TFE. Approximately three times as many males as females were killed by TFE 175 or 200 mg/kg i.p., between 12 and 48 h after injection.

This sex difference was only partially reversible following pretreatment with "opposite" sex hormones. Oestradiol pretreatment in males decreased toxicity insignificantly. In contrast, testosterone treatment in females increased toxicity significantly (P<0.02). The sex difference in TFE toxicity was maintained after pretreatment with phenobarbitone, but there was no increase in toxicity of TFE in either sex.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Sex</th>
<th>% dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>175</td>
<td>19</td>
<td>M</td>
<td>69</td>
</tr>
<tr>
<td>None</td>
<td>250</td>
<td>16</td>
<td>M</td>
<td>75*</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>250</td>
<td>17</td>
<td>M</td>
<td>55</td>
</tr>
<tr>
<td>Testosterone</td>
<td>250</td>
<td>20</td>
<td>F</td>
<td>70*</td>
</tr>
<tr>
<td>Phenobarbitone</td>
<td>175</td>
<td>20</td>
<td>M</td>
<td>65</td>
</tr>
<tr>
<td>Phenobarbitone</td>
<td>250</td>
<td>21</td>
<td>M</td>
<td>91</td>
</tr>
<tr>
<td>CCl₄</td>
<td>175</td>
<td>21</td>
<td>M</td>
<td>38</td>
</tr>
<tr>
<td>CCl₄</td>
<td>250</td>
<td>21</td>
<td>F</td>
<td>29</td>
</tr>
</tbody>
</table>

* Difference between control and testosterone treated females; P<0.02.

Carbon tetrachloride pretreatment did not abolish TFE toxicity. It caused a moderate, statistically insignificant, decrease in TFE toxicity in males and no change of toxicity in females.

DISCUSSION

Fluroxene

We believe that fluroxene toxicity in mice depends on its oxidative metabolism (Blake et al., 1967). Methyltestosterone increases drug-metabolizing enzymes in male and female rats, and untreated males show higher P-450 activity than do untreated females (Quinn, Axelrod and Brodie, 1958). If this applies to male mice, they should be more susceptible to a drug such as fluroxene whose toxicity depends on conversion to a toxic product. Our work shows that this is the case in Swiss Webster male mice. The dependence of the observed sex difference of fluroxene toxicity on inducible biotransformation is supported by the increase of toxicity after phenobarbitone pretreatment in both sexes. More mice of both sexes died after phenobarbitone pretreatment, but females showed a more dramatic response and became almost as susceptible as males. We believe that the conversion of fluroxene to a toxic metabolite can be stimulated to a maximum in both sexes. The test of this thesis, measurements of the rate and quantity of fluroxene biotransformation, was impossible because labelled fluroxene could not be obtained.

It can be postulated that testosterone treatment in females has the same inducing effect as occurs in males, whereas oestradiol, in the doses used in our experiments, suppressed the male hormone and allowed the microsomal enzymes to decrease.
SEX DIFFERENCES IN ANAESTHETIC TOXICITY:

The dependence of fluroxene toxicity on biotransformation in both sexes is supported further by the protective effect of a small dose of carbon tetrachloride. Carbon tetrachloride, in the dose used, depresses P-450 activity to approximately 25% of pre-treatment activity (Glende, 1972), thus presumably decreasing the total amount of toxic fluroxene metabolite.

These explanations base the sex difference of fluroxene toxicity on putative changes in enzyme activity. However, it is possible that the male sex hormone channels the biotransformation of fluroxene into a different and more toxic route. Fluroxene metabolites have been assayed qualitatively (and quantitatively) only in male mice (Blake et al., 1967). Comparative studies of metabolites in male and female mice may demonstrate whether different metabolites occur in the two sexes. Species differences of urinary fluroxene metabolites occur: man excretes mainly trifluoroacetate, while mice excrete mainly trifluoroethanol glucuronide (Gion et al., 1974).

The mechanism of postanaesthetic toxicity of fluroxene is unknown. We did not find specific lesions, detectable by light microscopy, in any of the vital organs of mice. One may speculate that interference with energy-transfer processes, for instance the coupling of oxygenation with phosphorylation, may be disturbed by intracellular metabolites of fluroxene.

Trifluoroethanol

The results of the TFE toxicity studies are not explained easily. We assumed that TFE would show, qualitatively, the same results as fluroxene and, quantitatively, a better reproducibility because of the better control of doses with i.p. injection compared with inhalation-time doses. Neither proved to be true.

The only similarity between fluroxene and TFE was a sex difference of toxicity of TFE in favour of females. Repeated attempts to quantify the ratio of TFE toxicity in males and females failed. We were unable to arrive at reproducible dose–response curves although we and others had previously determined LD_{50} of TFE to be about 350 mg/kg i.p. in male mice (Airaksinen and Tammisto, 1968; Blake et al., 1969; Ferstandig, L. L., unpublished results). The dose required to kill approximately 50% of the animals varied from 150 to 500 mg/kg and was unpredictable among batches of mice (table III). It is apparent, however, that males are more susceptible than females to TFE toxicity.

Therefore we determined a toxic dose of TFE for a given batch of mice and used this dose (175 and 250 mg/kg (table II)) to explore the effect of enzyme stimulation and depression by phenobarbitone and carbon tetrachloride pretreatment respectively, and the effect of “opposite” hormones.

Unlike fluroxene, TFE was not affected by pretreatment with phenobarbitone or carbon tetrachloride. We interpret this as an indication that the P-450 system is not primarily involved in the biotransformation of TFE. Either catalase or alcohol-dehydrogenase is more likely to be responsible for breakdown and toxicity of TFE, since we have shown previously that TFE toxicity may be decreased by the inhibition of catalase or dehydrogenase (Blake et al., 1969).

The only hormonal effect on TFE toxicity occurred in females after pretreatment with testosterone. The explanations for testosterone activity on fluroxene toxicity, discussed above, may apply here also. However, we speculate that some other enzyme system or target organ is the site of the TFE–testosterone interaction.

REFERENCES


**DIFFERENCES DANS LA TOXICITE DE L'ANESTHESIE SUIVANT LES SEXES: FLUROXENE ET TRIFLUOROETHANOL CHEZ LES SOURIS**

**RESUME**

On a trouvé une différence, suivant les sexes, dans la mortalité post-anesthésique après anesthésie par le fluroxène de souris Webster suisses. Davantage de mâles que de femelles y ont succombé. Cette toxicité dépend de la biotransformation, action qui peut être inversée par un prétraitement par les hormones du sexe opposé. La toxicité du trifluoroéthanol, métabolite du fluroxène, a été encore plus marquée sur les souris mâles, mais elle n'a été que partiellement influencée par les inhibiteurs ou stimulateurs d'enzymes microsomales ou par les hormones sexuelles.

**GESCHLECHTSUNTERSCHIEDE BEI DER NARKOSETOXIZITÄT: FLUROXEN UND TRIFLUROETHANOL BEI MÄUSEN**

**ZUSAMMENFASSUNG**

Ein Geschlechtsunterschied bei Anwendung von Fluroxen zeigte sich nach der Narkose bei Swiss Webster Mäusen. Männliche Mäuse waren mehr anfällig, als weibliche. Die Toxizität war biotransformationsbedingt und war nicht umkehrbar durch Vorbehandlung mit entgegengesetzten Geschlechtshormonen. Die Toxizität des Fluroxenmetaboliten "Trifluoroethanol" erwies sich ebenfalls als größer bei den Männchen, war aber teilweise durch mikrosomale Enzymhemmung, Reizung oder Geschlechtshormone beeinflusst.

**LAS DIFERENCIAS DE SEXO EN LA TOXICIDAD ANESTESICA: EL FLUROXENO Y EL TRIFLUOROETANOL EN LOS RATONES**

**SUMARIO**

Se encontró una diferencia de sexo en la mortalidad postanestésica después de anestesia de fluroxeno, en ratones Swiss Webster. Sucumbieron más machos que hembras. Esta toxicidad resultó dependiente de la biotransformación y se puede invertir mediante pretratamiento con hormonas del sexo "opuesto". La toxicidad del trifluoroetanol metabolito de fluroxeno estuvo también más marcada en los ratones machos, pero se vio solo parcialmente influenciada por inhibidores de enzima microsomales o estimuladores, o por hormonas sexuales.