Modification of Fluroxene Toxicity

H. F. Cascorbi, M.D., Ph.D.,* and A. V. Singh-Amaranath, M.D.†

Postanesthetic toxicity of fluroxene in Swiss Webster mice can be prevented by administration of enzyme inhibitors prior to anesthesia. Aminotriazole, a catalase inhibitor, pyrazole, an alcohol dehydrogenase inhibitor, and carbon tetrachloride, a P450 inhibitor, abolished postanesthetic death after an otherwise lethal dose of fluroxene. Disulfiram, an acetaldehyde oxidase inhibitor, reduced postanesthetic mortality. Ethanol also reduced postanesthetic mortality after fluroxene anesthesia. This indicates that trifluoroethanol, a metabolite of fluroxene, is responsible for postanesthetic death after fluroxene anesthesia in mice. (Key words: Fluroxene; Toxicity; Mice; Trifluoroethanol; Pyrazole; Aminotriazole; Disulfiram; Ethanol; Enzyme, microsomal.)

We have shown that fluroxene (Fluoromar) anesthesia may be followed by postanesthetic death in mice. The mortality rate, which depended on length of exposure to the anesthetic, was increased by pretreatment with phenobarbital and diminished by pretreatment with carbon tetrachloride, indicating that the capacity of the liver for metabolism of fluroxene was essential for the production of postanesthetic death after fluroxene anesthesia in mice.

We believed the toxic metabolite to be trifluoroethanol or one of its metabolites. We have shown that mice metabolize fluroxene to trifluoroethanol—glucuronide and to trifluoroacetate. Since trifluoroethanol has an LD₅₀ of 350 mg ip and its toxicity can be modified by treatment with agents which influence the metabolism of ethanol, we thought it would be interesting to study the effect of modifiers of alcohol metabolism on postanesthetic death after fluroxene anesthesia. In addition, we studied the effect of simultaneous ethanol administration on fluroxene toxicity.

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Received from the Department of Anesthesiology, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106. Accepted for publication December 13, 1972.

Methods and Materials

Male Swiss-Webster mice, 25–30 g, supplied by Carworth, New York, New York, were fed Purina Mouse Chow and water ad lib., and kept in an air-conditioned room. The mice were anesthetized in a 2.2-liter rubber-stoppered desiccator with inlet and outlet for anesthetic gases and a metal floor. Fluroxene was delivered from a Draeger “Vapor” halothane vaporizer calibrated for fluroxene by gas chromatography. The carrier gas was compressed air at a flow rate of 2 l/min.

The mice were anesthetized in groups of ten to 18 at a time. The animals were introduced into the desiccator; concentrations of fluroxene were monitored by gas chromatography. The desired concentrations were reached within 45 seconds after closure of the desiccator. The animals were anesthetized for two hours with fluroxene at 4.5 per cent unless otherwise stated.

Postanesthetic death was defined as any death within 30 hours of recovery from anesthesia. The animals were observed for 48 hours, and additional deaths occurring within this time were noted.

The following drugs and dosage schedules were used.

Carbon tetrachloride was given subcutaneously as a 20 per cent solution in Wesson Oil, 0.2 ml per mouse, 24 hours prior to exposure to anesthesia. 3-amino, 1,2,4-triazole (T. Aldrich Chemical) was given in a 4 per cent solution in water, 1,000 mg/kg body weight ip, two hours prior to anesthesia. Pyrazole (Eastman Kodak Company) was injected ip in a 1.5 per cent solution in water, 15 μg/kg body weight, just before induction of anesthesia. Ethanol, U.S.P., was diluted to 12.5 per cent with physiologic saline solution and given ip in 1,200 or 2,400 mg/kg doses. One dose was given 30 minutes before anesthesia, subsequent doses were administered every four hours. Disulfiram was shaken in 10 per cent acacia to make a 1 per cent suspension; 0.25 to
0.3 ml of this suspension was injected intraperitoneally every day for three days prior to anesthesia.

In order to assure that the bedding of our mouse cages did not influence hepatic metabolism, a shipment of 60 mice was divided into four equal groups. Two groups were kept in metal cages without bedding, the other two in metal cages with pine-shaving bedding. One of each two groups was anesthetized 24 hours after arrival, the other six days later.

### Results

The results of treatment with drugs modifying ethanol metabolism are shown in table 1. Aminotriazole, pyrazole, and carbon tetrachloride protected all animals against the postanesthetic death that occurred in control animals. Disulfiram reduced mortality to about a third of control after 30 hours and about half of control after 48 hours.

Table 2 shows the effects of administration of ethanol on mortality after two hours of fluroxene anesthesia. Depending on dose and dose schedule, ethanol had a marked to moderate protective effect. One or two doses of 2,400 mg/kg of ethanol, ip, reduced postanesthetic mortality to 10 to 25 per cent, compared with a death rate of 76 to 82 per cent of control animals.

Table 3 shows that the toxicity of two hours of fluroxene anesthesia increased markedly when the mice were kept on pine-shaving bedding. It also shows that the susceptibility of the mice to fluroxene toxicity decreased when they were kept in a metal cage for six days.

### Discussion

We believe the metabolism of fluroxene to be essential for the postanesthetic death of animals, since we found previously that induction of hepatic microsomal enzymes increases fluroxene toxicity. In this study we obtained indirect confirmation of the observation of Vesel et al. that cage environment, particularly bedding material, can be a strong enzyme-inducing stimulus.

Our tentative concept of fluroxene biotransformation is shown in figure 1. After the cleavage of the ether link, the vinyl moiety is converted to CO₂; the trifluoroethane is oxidized to trifluoroethanol; trifluoroethanol in

### Table 1. Effects of Pretreatment with Enzyme Inhibitors on Postanesthetic Death after Fluroxene Anesthesia in Mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number of Mice</th>
<th>24 Hours</th>
<th>30 Hours</th>
<th>48 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>50</td>
<td>60</td>
<td>76</td>
<td>82</td>
</tr>
<tr>
<td>3-Amino 1,2,4 triazole</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pyrazole</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Disulfiram</td>
<td>40</td>
<td>17</td>
<td>31.5</td>
<td>50</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* See text for dosage schedules.
† Hours after anesthesia.

### Table 2. Effects of Ethanol Administration on Postanesthetic Deaths after Fluroxene Anesthesia

<table>
<thead>
<tr>
<th>Dose Schedule</th>
<th>Number of Mice</th>
<th>24 Hours</th>
<th>30 Hours</th>
<th>48 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>50</td>
<td>60</td>
<td>76</td>
<td>82</td>
</tr>
<tr>
<td>1,200 mg/kg Q 4 H, 7 doses</td>
<td>15</td>
<td>20</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>2,400 mg/kg Q 4 H, 5 doses</td>
<td>13</td>
<td>23</td>
<td>23</td>
<td>38.5</td>
</tr>
<tr>
<td>2,400 mg/kg Q 4 H, 3 doses</td>
<td>12</td>
<td>0</td>
<td>8</td>
<td>34</td>
</tr>
<tr>
<td>2,400 mg/kg Q 4 H, 2 doses</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>2,400 mg/kg, 1 dose</td>
<td>16</td>
<td>0</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

* Hours after anesthesia.

### Table 3. Modification of Postanesthetic Deaths after Fluroxene Anesthesia by Cage Environment

<table>
<thead>
<tr>
<th>Environment</th>
<th>Number of Mice</th>
<th>24 Hours</th>
<th>30 Hours</th>
<th>48 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal grid, 24 hours</td>
<td>13</td>
<td>0</td>
<td>21</td>
<td>71</td>
</tr>
<tr>
<td>Pine bedding, 24 hours</td>
<td>15</td>
<td>6.5</td>
<td>13</td>
<td>76</td>
</tr>
<tr>
<td>Metal grid, 6 days</td>
<td>15</td>
<td>6.5</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Pine bedding, 6 days</td>
<td>17</td>
<td>20</td>
<td>71</td>
<td>100</td>
</tr>
</tbody>
</table>

* Hours after anesthesia.
Probable enzymes

\[
\text{CF}_3\text{CH}_2 - \text{O} \xrightarrow{\text{CF}_3\text{CH}_2\text{CO}_2} \text{CF}_3\text{CH}_2\text{CO}_2
\]

Inhibitors

\[
\text{CF}_3\text{CH}_2 = \text{O} \xrightarrow{\text{CF}_3\text{CO}_2} \text{CF}_3\text{CO}_2
\]

Fluoxetine

Carbon tetrachloride

P450 system

\[
\text{CF}_3\text{CH}_2\text{CO}_2 \xrightarrow{\text{to Glucuronide}} \text{CF}_3\text{CH}_2\text{CO}_2
\]

Trifluoroethanol

Microsomal ethanol oxidizing system [MEOS]

Alcohol dehydrogenase [ADH]

Catalase

\[
\text{CF}_3\text{CO}_2\text{H} \xrightarrow{\text{Distufiram}} \text{CF}_3\text{CO}_2\text{H}
\]

Trifluoroacetaldehyde

Acetaldehyde dehydrogenase

Carbon tetrachloride

Pyrazole

Aminotriazole

Trifluoroacetic acid

Fig. 1. Postulated pathways of fluoxetine biotransformation.

turn is converted into trifluoroacetaldehyde, and the final product is trifluoroacetate, which is excreted in the urine. Another pathway leads from trifluoroethanol via glucuronide transferase to trifluoroethanol–glucuronide, also a urinary excretion product. At least three pathways of trifluoroethanol to trifluoroacetaldehyde have been postulated. First, there is the microsomal ethanol-oxidizing system (MEOS), which is suppressed by carbon tetrachloride.

Second, catalase in the presence of a hydrogen-peroxide-generating system can catalyze the reaction from ethanol to acetaldehyde. This reaction is not specific for ethanol; methanol is apparently an equally good substrate. The large amount of catalase available in the body and the presence of various peroxide-generating systems make it reasonable to assume that some oxidation of ethanol occurs by this route. However, the evidence for this is controversial. Apparently 3-aminotriazole is not a specific inhibitor of catalase and is able to inhibit other drug-metabolizing enzyme systems as well. Therefore, the results of our studies do not prove that the catalase pathway is important in the biotransformation of trifluoroethanol to trifluoroacetaldehyde. At this time, we can only say that the administration of 3-aminotriazole reduces the toxicity of fluoxetine, presumably through depression of unspecified enzyme systems. However, the most important pathway from trifluoroethanol to trifluoroacetaldehyde is mediated by alcohol dehydrogenase (ADH), which is blocked by pyrazole. Our results show that pretreatment with any of the three enzyme inhibitors, carbon tetrachloride, aminotriazole, and pyrazole, will prevent postanesthetic mortality after two hours of fluoxetine anesthesia.
The interpretation of these findings is difficult. We like to think that the ADH (alcohol dehydrogenase) pathway is the major metabolic route of trifluoroethanol to trifluoroacetalddehyde. If this is true, inhibition of the catalase pathway or the MEOS pathway should be less effective than ADH inhibition. However, either treatment abolished postanesthetic mortality as completely as ADH inhibition. Maybe carbon tetrachloride inhibits not only the MEOS but also ADH sufficiently to reduce the production of trifluoroacetalddehyde from trifluoroethanol below toxic threshold levels. Further biochemical studies may clarify this problem.

It was surprising that disulfiram also decreased the postanesthetic toxicity of fluorozone anesthesia. Disulfiram is an inhibitor of acetaldehyde oxidase. It increases the amount of acetaldehyde present after ingestion of ethanol, producing the well-known antabuse reaction. Airaksinen et al. showed trifluoroethanol toxicity to increase after pretreatment with another acetaldehyde oxidase inhibitor, allopurinol. However, Blake et al. showed a decrease of trifluoroethanol toxicity with the same inhibitor. These contradictions were ascribed by Airaksinen to differences in dosage.

An increase in the toxicity of fluorozone anesthesia after pretreatment with disulfiram would implicate trifluoroacetalddehyde as the toxic metabolite of fluorozone. Decreased toxicity after disulfiram indicates to us that trifluoroacetalddehyde itself is not the toxic substance. Possibly a step from trifluoroacetalddehyde to trifluoroacetate is the potentially lethal event in the metabolism of fluorozone. Another explanation might be that disulfiram inhibits not only ADH but other enzyme systems as well. More detailed biochemical studies in vivo are necessary for the elucidation of this problem.

Ethanol competes for available enzyme with trifluoroethanol in vitro. This competition of trifluoroethanol and ethanol exists in vitro as well.

References