Glutathione Depletion Following Inhalation Anesthesia

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Glutathione depletion following inhalation of halogenated anesthetics was investigated as a possible mechanism of toxic reactions associated with anesthesia. Concentrations of reduced glutathione were measured in the blood, liver, lung and kidney of the mouse after anesthesia with enflurane, fluoroxene, halothane, isoflurane, methoxyflurane, or trichloroethylene. The anesthetic had no effect on glutathione concentrations in tissues except when fluoroxene was used. After two hours of fluoroxene anesthesia, glutathione in liver, lung, kidney, and blood was depleted by 96, 55, 85, and 61 per cent, respectively. The depletion was dose-dependent and was more extensive in animals anesthetized after phenobarbital pretreatment. Glutathione was also depleted in livers and lungs of rats anesthetized with fluoroxene (60 and 38 per cent, respectively). In blood of rhesus monkeys anesthetized with fluoroxene, glutathione was depleted by only 13 per cent. Extents of glutathione depletion are related to fluoroxene toxicities in the three species studied. (Key words: Metabolism: glutathione. Anesthetics, volatile: enflurane; fluoroxene; isoflurane; methoxyflurane; halothane; trichloroethylene.)

During clinical anesthesia, large doses of inhalation anesthetics (10–30 g) are administered to patients. Most of these agents are extensively metabolized, exceptions being isoflurane and enflurane. This imposes a large load on those enzymes and cellular constituents involved in metabolism and detoxification of drugs. This might result in important interactions with metabolism and detoxification of other drugs administered concurrently to surgical patients.

The important role of glutathione in protecting cells against drug toxicity is well established. Boyland has listed 17 groups of compounds that are detoxified by glutathione; among them are halogenated compounds and α,β-unsaturated carbonyl compounds. Mitchell et al. see the protective effect of glutathione as: 1) conjugation of drugs and their metabolites with glutathione followed by excretion of the respective mercapturic acids; 2) reversal of the SH-inhibition of certain enzymes; 3) protection of cellular membrane integrity by removal of lipid hydroperoxide and other peroxides generated by metabolism of drugs (hemolytic drugs as an example); 4) protection of vital nucleophilic sites in hepatocytes and other tissues from electrophilic attack by alkylating metabolites of drugs.

Glutathione involvement in detoxification may be anticipated from the chemical structures of the inhalation anesthetics (halogenated hydrocarbons, vinyl moiety of fluoroxene) and from observed extensive glutathione depletion in tissues of animals treated with some metabolites of the anesthetic agents, such as triluoroethanol and triluoroacetaldehyde, or fluoride, which inhibits glutathione synthesis in erythrocytes in vitro.

The only study of glutathione levels following exposure to inhalation anesthetics was reported by Brown et al., who observed glutathione depletion in tissues of rats anesthetized with chloroform but not those anesthetized with halothane. Glutathione was not even depleted by halothane anesthesia administered to enzyme-induced rats manifesting enhanced lipoperoxidation in the liver.

The present study was undertaken to determine glutathione concentrations in tissues of animals following anesthesia produced by various inhalation anesthetics.

Methods and Materials

The experimental animals were 170 female black C-57 mice (weight, 20 ± 3 g), 18 Sprague-Dawley female rats (weight 200 ± 13 g) and seven male rhesus monkeys (6 kg). The rodents were exposed to vapors of anesthetics in a 20-l exposure chamber. Air containing the desired vapor concentration flowed through the chamber at a rate of 5 l/min. Temperatures in the exposure chamber varied between 20 and 22 C. Concentrations of the anesthetic agents were measured by gas chromatography.

Those rodents referred to as “pretreated” received phenobarbital intraperitoneally five times 12 hours apart. Mice received a dose of 40 mg/kg in each treatment, rats 20 mg/kg. Exposure to the anesthetic drug followed 24 hours after the last administration. L-Cysteine-treated mice received l-cysteine in saline solution intraperitoneally in four doses of 150 mg/kg

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Received from the Departments of Anesthesiology and Surgery, University of Miami School of Medicine, Miami, Florida 33132.

Accepted for publication December 19, 1977. Supported in part by USPHS grant GM 16498 and NIH GM 01714.

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Table 1. Glutathione Concentrations (mg/g Wet Tissues) in Control Mice and in Mice after Two-hour Anesthesia (Means ± SD; n = 4)

<table>
<thead>
<tr>
<th>Glutathione</th>
<th>Concentration (Per Cent)</th>
<th>Liver Control</th>
<th>Liver Exposed</th>
<th>Kidney Control</th>
<th>Kidney Exposed</th>
<th>Lung Control</th>
<th>Lung Exposed</th>
<th>Blood Control</th>
<th>Blood Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoroxene</td>
<td>0.75</td>
<td>1.75 ± .01</td>
<td>.13 ± .12*</td>
<td>.185 ± .006</td>
<td>.027 ± .005*</td>
<td>.150 ± .027</td>
<td>.022 ± .005*</td>
<td>.276 ± .029</td>
<td>.106 ± .018*</td>
</tr>
<tr>
<td>Halothane</td>
<td>0.75</td>
<td>1.44 ± .38</td>
<td>1.29 ± .87</td>
<td>.200 ± .020</td>
<td>.185 ± .010</td>
<td>.175 ± .034</td>
<td>.192 ± 0.10</td>
<td>.291 ± .026</td>
<td>.138 ± .044</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>0.75</td>
<td>1.58 ± .16</td>
<td>1.30 ± .20</td>
<td>.152 ± .033</td>
<td>.162 ± .033</td>
<td>.162 ± .033</td>
<td>.157 ± .027</td>
<td>.249 ± .031</td>
<td>.261 ± .029</td>
</tr>
<tr>
<td>Methoxyflurane</td>
<td>0.75</td>
<td>1.40 ± .37</td>
<td>1.38 ± .74</td>
<td>.197 ± .036</td>
<td>.172 ± .046</td>
<td>.240 ± .028</td>
<td>.240 ± .050</td>
<td>.271 ± .045</td>
<td>.277 ± .079</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>0.75</td>
<td>1.53 ± .20</td>
<td>1.33 ± .15</td>
<td>.235 ± .041</td>
<td>.207 ± .007</td>
<td>.247 ± .036</td>
<td>.240 ± .060</td>
<td>.253 ± .021</td>
<td>.264 ± .034</td>
</tr>
<tr>
<td>Enflurane</td>
<td>0.75</td>
<td>1.64 ± .17</td>
<td>1.45 ± .20</td>
<td>.187 ± .002</td>
<td>.170 ± .045</td>
<td>.205 ± .038</td>
<td>.247 ± .046</td>
<td>.253 ± .037</td>
<td>.214 ± .043</td>
</tr>
</tbody>
</table>

* Significant difference between exposed and the control group, unpaired t-test, P < 0.01.

Each, 12 hours prior to exposure, immediately before and after exposure, and two hours after exposure.

Monkeys were anesthetized for four hours with fluoroxene, 5.5 per cent. The details of the experimental conditions were reported by Munson et al.*

The rodents were sacrificed by decapitation and blood, liver, kidney, and lung were analyzed for glutathione and nonvolatile fluoride. Samples of mouse liver and kidney were removed immediately after death and were fixed in formaldehyde, 10 per cent, for histologic examination. Glutathione was determined by a fluorometric assay described by Cohn and Lyle* using the Amino-Bowman spectrophotofluorometer. Concentrations of glutathione in the reduced form only are measured by this method. Nonvolatile fluoride in tissues and blood was determined in samples alkalized with five drops of 3N NaOH, dried at 105°C for 24 hours and ground. A weighed amount of dry sample was wrapped in black ignitor paper, wetted with one drop of octyl alcohol and combusted in a Thomas Ogg combustion flask containing oxygen and 10 ml of 5 per cent EDTA; fluoride was measured in EDTA solution at pH 6.5 by a specific fluoride ion electrode. Concentrations are expressed as µg/g wet tissue. The method is reliable for fluoride concentrations in tissues of 2 µg/g or more.

Six groups of experiments were performed.

Twenty-four mice (Group 1) were exposed for two hours to anesthetic concentrations of one of the following agents (in percentages): enfurane, 1.3; fluoroxene, 3%; halothane, .75%; isoflurane, 1.1; methoxyflurane, 4; trichloroethylene, 4. Four mice were anesthetized simultaneously with one drug and four unexposed animals in each study served as controls. Twenty mice were exposed for two hours to fluoroxene, 0.8 per cent (Group 2). Ten of the mice were pretreated with phenobarbital. Four non-exposed animals (two unpretreated and two pretreated) and four animals exposed to fluoroxene (two unpretreated and two pretreated) were sacrificed according to the following time schedule: immediately at the end of exposure, and 5, 10, 24, and 48 hours after the exposure.

Twelve mice, treated with 1-cysteine, were exposed to fluoroxene, 1 per cent, simultaneously with 12 untreated animals (Group 3). No spontaneous death occurred. Four 1-cysteine treated animals, four untreated animals, and four unexposed animals were sacrificed at the end of exposure, and five and 24 hours later.

Thirty-six mice were pretreated with phenobarbital (Group 4). Six pretreated animals served as controls; 18 animals were treated with 1-cysteine. Twelve animals treated with 1-cysteine were exposed to fluoroxene, 1 per cent, simultaneously with 12 animals that received only phenobarbital. Four animals from each group were sacrificed at the end of exposure. The times of spontaneous deaths of the rest of the animals were recorded.

Twelve rats were anesthetized simultaneously with fluoroxene, 3 per cent, and six animals served as controls (Group 5). Six of the exposed animals and three of the controls were pretreated with phenobarbital. Three pretreated and three unpretreated animals were sacrificed immediately at the end of anesthesia; the rest of the animals were sacrificed five hours thereafter.

Blood samples collected prior to anesthesia and at the end of anesthesia from five monkeys were analyzed for glutathione (Group 6). Blood samples from six mice pretreated with phenobarbital and anesthetized with fluoroxene were collected prior to anesthesia and after anesthesia.

Glutathione levels are presented as percentages of mean concentrations measured in tissues of animals used as controls in the same experiment whose tissues were analyzed simultaneously with those from the experimental group. Thus, any genetic or environment effect on glutathione levels could be minimized. The t test for nonpaired data was used to establish significance between mean glutathione concentrations in experimental and control groups.
Results

Phenobarbital pretreatment of L-cysteine treatment did not affect glutathione concentrations in blood and tissues. The nonvolatile fluorine concentrations in tissues of control animals were less than 2 μg/g wet tissue. Histologic examination of livers and kidneys of control animals did not show any pathologic change.

Glutathione depletion in mouse tissues after two hours of anesthesia was statistically significant only when floroxene was used (table 1). Glutathione was depleted to 7 per cent in the livers of mice anesthetized with floroxene; in lung and kidney it was depleted to 15 per cent of the original values, and in blood to 39 per cent. One mouse died during anesthesia. Glutathione concentrations in tissues of mice anesthetized with other agents were not statistically different from those of the respective control groups.

Glutathione was depleted in all tissues at the end of exposure to floroxene, 0.8 per cent, and returned slowly to normal (fig. 1). Liver glutathione recovered fastest, in less than five hours; blood glutathione recovered slowest, in more than 48 hours. Glutathione depletions in all tissues were more pronounced in mice pretreated with phenobarbital. No mouse pretreated with phenobarbital survived ten hours after exposure. Nonvolatile fluorine concentrations in tissues of unpretreated mice were increased, and peaked ten hours or less after exposure (fig. 2). The fluorine concentrations in all tissues of animals pretreated with phenobarbital, were higher than corresponding concentrations in unpretreated animals (approximately triple), and peak concentrations were reached approximately ten hours after exposure. The exception was liver, in which the peak concentration was reached at the end of anesthesia and was lower than peak concentrations in other tissues.

L-Cysteine administration did not affect normal glutathione concentrations in tissues. Following exposure to floroxene, L-cysteine treatment hastened glutathione recovery in liver (fig. 3), delayed the deaths of animals pretreated with phenobarbital (fig. 4), and decreased nonvolatile fluorine concentrations in the liver (fig. 5).

Histologic examination showed hepatocellular changes in all exposed mice in Groups 3 and 4; all kidneys were normal. The predominant findings in

Fig. 1. Effects of phenobarbital pretreatment on glutathione levels in mouse tissues following a two-hour exposure to 0.8 per cent floroxene. Bars represent ratios of the mean concentrations in tissues of experimental animals over the controls, expressed as percentages. Means are calculated from individual values measured in tissues of two animals. The difference between the values never exceeded 7 per cent of the mean value. Numbers under the bars indicate time intervals (in hours) between end of exposure and sacrifice of animals. † indicates that all animals were dead.
the livers were centrilobular vacuolization, moderate fatty metamorphosis, passive hyperemia, and sinusoidal congestion. These changes progressed after exposure and were most severe in the livers of mice sacrificed 24 hours after exposure. Extents of damage varied among groups according to treatment. At the same exposure the damage was more severe in phenobarbital-pretreated mice than in unpretreated animals. In mice treated with L-cysteine, hepatic damage was less extensive than that in untreated mice. In contradiction to mice without L-cysteine treatment, the damage in cysteine-treated mice was most apparent in mice sacrificed five hours after exposure and was less in mice sacrificed at 24 hours.

Flurxene anesthesia was not fatal to rats. Glutathione was depleted less in rat tissues than in mouse tissues, liver and lung being affected the most (fig. 6). Pretreatment with phenobarbital did not influence depletion or rate of recovery as much as in mice. Glutathione depletion was also observed in blood of all monkeys anesthetized with flurxene, and the decrease of glutathione concentration was significant when tested by t test for paired data. Extents of depletion were the same in unpretreated and pretreated monkeys: from 0.20 mg/ml ± 0.007 (SD) to 0.156 mg/ml ± 0.060 at the end of anesthesia in unpretreated monkeys and to 0.148 mg/ml ± 0.034 at death of pretreated monkeys.

**Discussion**

In this study glutathione concentrations were measured in blood, liver, lung and kidney of the mouse after anesthesia with enflurane, flurxene, halothane, isoflurane, methoxyflurane, and trichloroethylene. The anesthetic had no effect on glutathione concentrations in tissues except when flurxene was used. Glutathione depletion following flurxene anesthesia appeared to be related to the extent of flurxene metabolism and to its toxicity. Phenobarbital pretreatment enhanced metabolism of flurxene, as demonstrated by increased concentra-
Fig. 4. Effects of L-cysteine treatment on mortality of mice pre-treated with pentoanalgetic and exposed for two hours to 1 per cent fluoroxene. Eight animals received only pentoanalgetic and fluoroxene (open bars). Eight animals also received L-cysteine treatment (striped bars). Bars represent percentages of dead animals in each group at the intervals after the end of exposure indicated on the abscissa.

In the liver, fluoroxene can be converted to fluoroxene poisoning is caused by the formation of a toxic metabolite, trifluoroacetate, which is further metabolized to trichloroacetic acid (TFAA) and trichloroacetaldehyde. The toxic effects of these compounds are mediated by their interaction with thiol-containing enzymes, leading to the depletion of glutathione, an important antioxidant.

Glutathione depletion in mouse liver and erythrocytes following TFE or trifluoroacetaldehyde administration has been demonstrated by Rosenberg. Both these compounds are very toxic. Since the toxicity of fluoroxene is decreased by all inhibitors of enzymes catalyzing alcohol oxidation and by drugs binding trifluoroacetate, it appears that TFAA toxicity is slight. The toxicity-limiting factor in fluoroxene metabolism is trifluoroacetate formation or metabolism. Trifluoroacetate is toxic to animals and is not excreted by tissues of animals anesthetized with halothane, it is not likely that TFE or trifluoroacetaldehyde is formed from halothane. It is more likely that TFAA is formed from halothane by a completely different metabolic pathway, as suggested by Cohen et al.

The mechanism of glutathione depletion following exposure to fluoroxene involves the detoxification of fluoroxene to trifluoroacetaldehyde (TFA). Trifluoroacetaldehyde is further metabolized to trifluoromethanol (TFM) and trifluoroacetic acid (TFAA), which are toxic to tissues. Glutathione depletion in organs and tissues following treatment with L-cysteine has been observed, indicating that L-cysteine treatment can help counteract the toxic effects of fluoroxene.

Fig. 5. Effects of L-cysteine treatment on concentrations of nonvolatile fluorinated compound in liver and muscle of mice exposed for two hours to 1 per cent fluoroxene. Intervals between end of exposure and sacrifice of animals are on the abscissa. Open bars represent animals without treatment; striped bars represent animals that received L-cysteine.
GLUTATHIONE DEPLETION FOLLOWING INHALATION ANESTHESIA

Fig. 6. Glutathione levels in rat tissues following two-hour anesthesia with 3 per cent fluoroxyne. Bars represent the ratios of mean concentrations in tissues of three experimental animals over means for three controls, expressed as percentages. Coefficients of variation within the groups of three did not exceed 12 per cent of the means. White bars represent animals sacrificed at the end of exposure and dotted bars, animals sacrificed five hours after exposure. Unpretreated means animals receiving only fluoroxyne; pretreated means animals pretreated with phenobarbital prior to exposure to fluoroxyne.

Fluoroxyne anesthesia remains unexplained. The enhanced washout of fluorinated metabolites from the livers of our mice treated with L-cysteine and less extensive hepatic damage in these mice suggests that some small amount of fluoroxyne is detoxified in the liver by conjugation with glutathione. L-Cysteine had a similar protective effect in mice receiving injections of TFE or trifluoracetaldehyde.31 The protective effect was less than demonstrated for other drugs that form mercapturic acid,32 such as bromobenzene33 or acetyaminophen.4 If the massive glutathione depletion observed in our study were caused by conjugation of fluoroxyne or its metabolite with glutathione, large amounts of a corresponding mercapturic acid would be excreted in urine. However, careful study with labeled fluoroxyne in mice16 and our quantitative study in man25 indicate that less than 20 per cent of urinary radioactivity or fluorine may be attributed to metabolites other than TFAA, TFE and TFE-glucuronide. Therefore, it is speculated that the extensive glutathione depletion in blood and tissues following fluoroxyne anesthesia is the result of perturbation of glutathione status in the cells, caused probably by the trifluoroethyl moiety of fluoroxyne rather than by a conjugation reaction.

The profound glutathione depletion following fluoroxyne anesthesia, as shown in these animal studies, may contribute to toxicity and to the recently demonstrated mutagenicity of fluoroxyne,26 and may modify the toxicities of other drugs administered at the same time. As a result of glutathione depletion, membranes of cells may be damaged by disorders in the balance of oxidative and reductive processes in cells. The hemorrhagic disorders caused by fluoroxyne anesthesia8,27 may result from such a mechanism.

However, more information is needed before transfer of data to man because of species differences in reactions of fluoroxyne anesthesia.28

The authors thank Paul Dolman, B.S., Martha Dominech, B.S., and Steve Vachetti, B.S., for excellent technical assistance.

References

11. Yoshimura N, Fiserova-Bergerova V, Holaday DA: Relation-
ship between trifluoroethanol and fluoxetine toxicity in mice. Anesthesia 23:835–841, 1974 (Japanese)
22. Van Dyke RA, Gandolfi AJ: Studies on irreversible binding of radioactivity of (14C) halothane to rat hepatic microsomal lipids and proteins. Drug Metab Dispos 2:469–476, 1974

Cardiac Physiology

PROPRANOLOL, NITROGLYCERIN AND CORONARY-ARTERY DISEASE Propranolol and nitroglycerin appear to have opposite effects on some aspects of left ventricular performance. Propranolol increases left ventricular volume, while nitroglycerin reduces it. Propranolol slows cardiac rate; an increased rate is often associated with the use of nitroglycerin. The authors have studied both drugs singly and in combination in 18 patients with coronary-artery disease. Nitroglycerin (0.4 mg sublingually) decreased left ventricular end-diastolic volume index (LVEDVI [ml/m²]) from 94 ± 8 (SEM) to 66 ± 5. Nitroglycerin administration increased left ventricular ejection fraction (LVEF) from 0.52 ± 0.04 to 0.64 ± 0.05. Cardiac rate increased significantly from 72 ± 2 to 79 ± 79/min. Two hours after oral administration of propranolol (20 mg), cardiac rate had changed significantly (63 ± 2/min); no significant change in LVEDVI or LVEF was observed. When the dosage of propranolol was increased to 40 mg, there was a significant increase in LVEDVI (113 ± 4 ml/m²) and a decrease in LVEF (0.47 ± 0.06). Cardiac rate decreased significantly (63 ± 2). Propranolol (80 mg) produced similar changes in LVEDVI (90 ± 6 ml/m²), LVEF (0.58 ± 0.03), and rate (54 ± 2/min). When therapy with both propranolol (40 mg) and nitroglycerin was instituted, LVEDVI was decreased (59 ± 3 ml/m²) significantly compared with control. A significant change in LVEF was not observed. Combination of propranolol (80 mg) and nitroglycerin produced a significant decrease in LVEDVI (58 ± 4 ml/m²) and a significant increase in LVEF (0.75 ± 0.02) compared with control. Cardiac rates with combination therapy were 64 ± 3/min (propranolol, 40 mg) and 51 ± 2/min (propranolol, 80 mg); the latter value represented a statistically significant change. The authors conclude that the data suggest that combination therapy with nitroglycerin and propranolol may “favorably alter the left ventricular volume and heart rate in terms of the myocardial demand for oxygen.” (Steele PP, and others, Effects of propranolol and nitroglycerin on left ventricular performance in patients with coronary arterial disease. Chest 73:19–23, 1978.)