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, halothane, isoflurane, and trichlorethylene. The anesthetic had no effect on glutathione concentrations in tissues except when halothane was used. After two hours of halothane anesthesia, glutathione in liver, lung, kidney, and blood was depleted by 93, 85, 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 enflurane (60 and 38 per cent, respectively). In blood of rhesus monkeys anesthetized with enflurane, glutathione was depleted by only 13 per cent. Extents of glutathione depletion are related to halothane toxicities in the three species studied. (Key words: Metabolism: glutathione. Anesthetics, volatile: enflurane; halothane; isoflurane; methoxyflurane; halothane; trichloroethylen.)

During clinical anesthesia, large doses of inhalation anesthetics (10–30 g) are administered to patients. Most of these agents are extensively metabolized, excepting 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-unsatuated 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 enflurane) and from observed extensive glutathione depletion in tissues of animals treated with some metabolites of the anesthetic agents, such as trilfluoroacetone and trifluoroacetone, 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.
GLUTATHIONE DEPLETION FOLLOWING INHALATION ANESTHESIA

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>Substance</th>
<th>Concentration (Per Cent)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lung</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Exposed</td>
<td>Control</td>
<td>Exposed</td>
<td>Control</td>
</tr>
<tr>
<td>Fluorexone</td>
<td>3</td>
<td>1.75 ± 0.01</td>
<td>1.13 ± 0.02*</td>
<td>1.83 ± 0.006</td>
<td>.027 ± .005*</td>
</tr>
<tr>
<td>Halothane</td>
<td>0.75</td>
<td>1.44 ± 0.38</td>
<td>1.29 ± 0.07</td>
<td>1.00 ± .020</td>
<td>1.85 ± 0.010</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>0.4</td>
<td>1.58 ± 0.16</td>
<td>1.30 ± 0.20</td>
<td>1.12 ± 0.003</td>
<td>1.10 ± 0.003</td>
</tr>
<tr>
<td>Methoxyflurane</td>
<td>0.6</td>
<td>1.40 ± 0.37</td>
<td>1.38 ± 0.74</td>
<td>1.17 ± 0.036</td>
<td>1.72 ± 0.046</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1.1</td>
<td>1.33 ± 0.20</td>
<td>1.33 ± 0.15</td>
<td>1.23 ± 0.041</td>
<td>1.207 ± 0.097</td>
</tr>
<tr>
<td>Enflurane</td>
<td>1.3</td>
<td>1.64 ± 0.17</td>
<td>1.45 ± 0.20</td>
<td>1.187 ± 0.002</td>
<td>1.170 ± 0.045</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 fluorexone, 5.5 per cent. The details of the experimental conditions were reported by Munson et al.8

The rodents were sacrificed by decapitation and blood, liver, kidney, and lung were analyzed for glutathione and nonvolatile fluorine. 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 Lyle8 using the Amino-Bowman spectrophotofluorometer. Concentrations of glutathione in the reduced form only are measured by this method. Nonvolatile fluorine in tissues and blood was determined in samples alkalinized with five drops of 3 N 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 fluorine 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): enflurane, 1.3; fluorexone, 3; halothane, 75; iso-flurane, 1.1; methoxyflurane, 6; 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 fluorexone, 0.8 per cent (Group 2). Ten of the mice were pretreated with phenobarbital. Four nonexposed animals (two pretreated and two pretreated) and four animals exposed to fluorexone (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 t-cysteine, were exposed to fluorexone, 1 per cent, simultaneously with 12 untreated animals (Group 3). No spontaneous death occurred. Four t-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 t-cysteine. Twelve animals treated with t-cysteine were exposed to fluorexone, 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 fluorexone, 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 monkeys pretreated with phenobarbital and anesthetized with fluorexone 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 environmental effects 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 fluroxene was used (table 1). Glutathione was depleted to 7 per cent in the livers of mice anesthetized with fluroxene; 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 fluroxene, 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 untreated 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 untreated 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 fluroxene, 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
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 contradistinction 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.

Fluroxene 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 fluroxene, 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 pre-

treated monkeys: from 0.20 mg/ml ± 0.007 (SD) to 0.156 mg/ml ± 0.006 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, fluroxene, halothane, isoflurane, methoxyflurane, and trichloroethylene. The anesthetic had no effect on glutathione concentrations in tissues except when fluroxene was used. Glutathione depletion following fluroxene anesthesia appeared to be related to the extent of fluroxene metabolism and to its toxicity. Phenobarbital pretreatment enhanced metabolism of fluroxene, as demonstrated by increased concentra-
tions of nonvolatile fluorine in tissues, glutathione depletion, and mortality in some animal species. We found a relationship between the extent of glutathione depletion and fluorocone toxicity in mice, rats, and rhesus monkeys. In mice, fluorocone anesthesia is fatal\(^{10,11}\) and glutathione depletion in tissues is extensive. Unpretreated monkeys tolerate fluorocone anesthesia well\(^a\) and glutathione levels in their blood are depleted slightly (approximately 13 per cent). Fluorocone toxicity\(^{12}\) and extent of glutathione depletion in the rat are between those in the mouse and the monkey.

The fluorocone molecule is an ether composed of two moieties, the vinyl group and the trifluoroethyl group, each of which is known to deplete glutathione in vivo.\(^{5,6}\) As a result of the detoxification of \(\alpha,\beta\)-unsaturated carbonyl compounds of conjugation with glutathione, glutathione is depleted from tissues and the corresponding mercapturic acids are excreted.\(^1\) The binding of the vinyl group in the fluorocone molecule to cytochrome P-450, followed by destruction of the heme of cytochrome P-450, has been demonstrated by Ivanetich et al.\(^{13-15}\) The trifluoroethyl group is oxidized to trifluoroethanol (TFE) and trifluoroacetic acid (TFAA), trifluoroacetaldehyde being a postulated intermediate.\(^{16}\) Glutathione depletion in mouse liver and erythrocytes following TFE or trifluoroacetaldehyde administration has been demonstrated by Rosenberg.\(^{2}\) Both these compounds are very toxic.\(^{11,17-19}\) Since the toxicity of fluorocone is decreased by all inhibitors of enzymes catalyzing alcohol oxidation and by drugs binding trifluoroacetaldehyde,\(^{10-21}\) and since TFAA toxicity is slight,\(^{17,18}\) the toxicity-limiting factor in fluorocone metabolism is trifluoroacetaldehyde formation or metabolism. Trifluoroacetaldehyde has been alleged to be formed from halothane, and then oxidized to TFAA.\(^{21,22}\) Since halothane anesthesia is not fatal to experimental animals, and since glutathione is not depleted from 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.\(^{23}\)

The mechanism of glutathione depletion following...
fluroxene 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 fluroxene is detoxified in the liver by conjugation with glutathione. l-Cysteine had a similar protective effect in mice receiving injections of TFE or trifluoroacetaldehyde. The protective effect was less than demonstrated for other drugs that form mercapturic acid, such as bromobenzene or acetaminophen. If the massive glutathione depletion observed in our study were caused by conjugation of fluroxene or its metabolite with glutathione, large amounts of a corresponding mercapturic acid would be excreted in urine. However, careful study with labeled fluroxene in mice and our quantitative study in man indicate that less than 20% of urinary radioactivity or fluoride 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 fluroxene anesthesia is the result of perturbation of glutathione status in the cells, caused probably by the trifluoroethyl moiety of fluroxene rather than by a conjugation reaction.

The profound glutathione depletion following fluroxene anesthesia, as shown in these animal studies, may contribute to toxicity and to the recently demonstrated mutagenicity of fluroxene, 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 fluroxene anesthesia may result from such a mechanism.

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

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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 ± 7/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 ± 0.08). 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 increase 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.)