Halothane-induced Hepatic Necrosis in Triiodothyronine-pretreated Rats

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Hepatic centrilobular necrosis developed in rats pretreated with triiodothyronine (T3) and then anesthetized with halothane, 1 per cent, for two hours at an ambient oxygen concentration. Increasing oxygen concentrations decreased the severity of the lesion, there being a significantly (P < 0.05) less severe lesion with oxygen, 99 per cent, as compared with 21 per cent. Pretreatment with phenobarbital alone resulted in hepatic necrosis only when hypoxia (F2O2 0.14) was also present, and there was no significant worsening of the T3-induced lesion when phenobarbital was added at any oxygen concentration studied. However, the lesion produced by T3 and oxygen, 14 per cent, was significantly worse than the lesion produced by phenobarbital and oxygen, 14 per cent. Glutamic pyruvic transaminase (SGPT) was significantly elevated to 776 (±226) U/l in the T3-treated rats (10 mg/kg/day, orally) immediately after halothane anesthesia. There was a significant decrease in glutathione to 1.48 (±0.06) mg/g liver 24 hours after T3 administration (1 mg/kg subcutaneously for five days), but no further decrease with continued T3 pretreatment or with halothane anesthesia. Pretreatment with T3 caused a significant decrease in cytochrome P-450 to 0.41 (±0.01) nmol/mg microsomal protein, and halothane anesthesia caused a further significant decrease to 0.27 (±0.04) nmol/mg microsomal protein. The mechanism for the hepatic toxicity of halothane in this model remains to be determined. (Key words: Anesthetics, volatile; halothane. Biotransformation: enzyme induction. Hormones: thyroid. Hypnotics, barbiturates: phenobarbital. Hypoxia. Induction, enzyme. Liver: hepatotoxicity; metabolism; microsomes. Toxicity, hepatic.)

It is generally accepted that hepatic necrosis may follow the administration of halothane, but the mechanism of such injury remains speculative. The irreversible binding of halothane intermediate metabolites to macromolecules in the liver may be related to halothane hepatotoxicity.1-4 Widger et al.4 found in rats pretreated with phenobarbital and anesthetized with halothane that hypoxia increased covalent binding of 14C-halothane metabolites to microsomal lipids more than threefold. They concluded that the products of the reductive metabolic pathway for halothane are potentially more hepatotoxic than the oxidative halothane metabolites, and in addition that defluorination of halothane occurs under hypoxic conditions. Further, pretreatment with potent enzyme inducers, such as polychlorobiphenyls, with halothane anesthesia, even at high oxygen concentrations, may cause centrilobular hepatic necrosis in rats.4

Thyroxyne has been shown to enhance the hepatic damage caused by carbon tetrachloride, whereas propylthiouracil and thyroidectomy to some extent protect against the development of the lesions.5,6 In addition, there is evidence that drug-metabolizing ability is increased in hyperthyroid patients,7 and we were therefore interested in determining the role of thyroid function, and triiodothyronine (T3) in particular, in the production of halothane hepatotoxicity.

Methods and Materials

Groups of four to six male Sprague-Dawley rats (75-125 g)† ‡ ± were treated with T3 in 5 per cent Tween 80* in water, in doses ranging from 1 to 10 mg/kg, orally, for six days, or with T3 in 5 per cent Tween 80 in physiologic saline solution, 1 mg/kg, subcutaneously (sc), for five days. Similar numbers of rats were treated with Tween 80 alone, administered either orally or subcutaneously. Twenty-four hours after pretreatment, groups of rats (four to six) were anesthetized with halothane (1 per cent) for two hours at oxygen concentrations that were ambient (21 per cent), low (14 per cent) or high (99 per cent). Another series of groups of rats (four to six in each group) received similar inspired oxygen concentrations without halothane. These experiments were carried out separately for all the methods of pretreatment—T3, 1 mg/kg/day, 5 mg/kg/day, and 10 mg/kg/day, orally for six days; T3, 1 mg/kg/day, sc, for five days; Tween 80 either orally or subcutaneously. In addition, groups of four to six rats were pretreated with phenobarbital, 75 mg/kg, intraperitoneally (ip) for three days, and similar numbers of control animals received saline injections ip for three days. Also, groups of T3-pretreated rats (1 mg/kg, sc, for five days) received phenobarbital, 75 mg/kg/day, ip, for three days or

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Received from the Departments of Anesthesiology and Pharmacology, Vanderbilt University, Nashville, Tennessee 37232. Accepted for publication December 14, 1979. Supported by United States Public Health Service Grant No. GM 15431.

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HALOTHANE TOXICITY IN T₃-TREATED RATS

Table 1. Triiodothyronine (T₃) Levels in Plasmas of T₂-pretreated Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T₃ (µg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>90</td>
</tr>
<tr>
<td>Tween 80, oral, 1 day</td>
<td>95 ± 15</td>
</tr>
<tr>
<td>Tween 80, oral, 6 days</td>
<td>160</td>
</tr>
<tr>
<td>Tween 80, oral, 6 days post-halothane</td>
<td>118 ± 8</td>
</tr>
<tr>
<td>T₃, 1 mg/kg, oral, 1 day</td>
<td>863 ± 398</td>
</tr>
<tr>
<td>T₃, mg/kg, oral, 6 days</td>
<td>2,725 ± 575</td>
</tr>
<tr>
<td>T₃, 10 mg/kg, oral, 6 days</td>
<td>30,000</td>
</tr>
<tr>
<td>T₃, 1 mg/kg, sc, 5 days</td>
<td>2,130 ± 270</td>
</tr>
<tr>
<td>T₃, 1 mg/kg, oral, 5 days post-halothane</td>
<td>800 ± 200</td>
</tr>
<tr>
<td>T₃, 10 mg/kg, oral, 6 days post-halothane</td>
<td>4,250 ± 950</td>
</tr>
</tbody>
</table>

* Tween 80 = vehicle for T₃.

Table 2. Net Increases in Body Weight (Mean ± SEM) of Rats Pretreated with Triiodothyronine (T₃)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Net Increase (g)</th>
<th>Per Cent Decrease from Control Value at End of Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral administration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 80, control (n = 451)</td>
<td>29 ± 1</td>
<td></td>
</tr>
<tr>
<td>T₃, 10 mg/kg/day, 6 days (n = 15)</td>
<td>10 ± 2*</td>
<td>67</td>
</tr>
<tr>
<td>T₃, 5 mg/kg/day, 6 days (n = 15)</td>
<td>9 ± 2*</td>
<td>68</td>
</tr>
<tr>
<td>T₃, 1 mg/kg/day, 6 days (n = 15)</td>
<td>15 ± 1*</td>
<td>47</td>
</tr>
<tr>
<td>Subcutaneous administration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 80, control (n = 30)</td>
<td>27 ± 2</td>
<td></td>
</tr>
<tr>
<td>T₃, 1 mg/kg/day, 5 days (n = 15)</td>
<td>4 ± 2*</td>
<td>85</td>
</tr>
<tr>
<td>T₃, 1 mg/kg/day, 5 days, + phenobarbital, 5 mg/kg, ip, 3 days (n = 15)</td>
<td>10 ± 2*</td>
<td>64</td>
</tr>
</tbody>
</table>

* P < 0.05 compared with control.
† n = number of rats.

The effect of pretreatment with T₃ (1 mg/kg, sc, for five days) and halothane (1 per cent in oxygen, 21 per cent) on hepatic glutathione (GSH) was determined in groups of rats (five in each group: total number = 25) before T₃ treatment, after 24 hours of T₃ treatment, immediately before halothane anesthesia, and six hours and 24 hours after halothane anesthesia. Control experiments were also carried out simultaneously on rats pretreated with Tween 80 (five in each group: total number = 20 rats). Cytochrome P-450 estimations were carried out in groups of three rats (total number = 24) pretreated with T₃ (1 mg/kg, sc, for five days), before and immediately and 24 hours after anesthesia with halothane, 1 per cent, in oxygen, 21 per cent. Further control groups of six rats (total

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Saline injections, ip, for three days. After pretreatment in the manner described above, rats were anesthetized with 1 per cent halothane for two hours at an oxygen concentration of 21, 14, or 99 per cent. Further control groups of rats received similar oxygen concentrations without halothane.

The animals were anesthetized in a 60-l glass-sided aquarium with an inlet port and an outlet port at opposite ends. Halothane, vaporized using a Fluotec vaporizer,† † was administered at a concentration of 1 per cent in nitrogen with oxygen, 21 or 14 per cent, or in oxygen, 99 per cent, for two hours at a flow rate of 10 l/min. The oxygen concentration within the chamber was continuously monitored using a polarographic oxygen analyzer,§§ and at 15-minute intervals samples from within the chamber were analyzed for halothane concentration by gas chromatography.

Development of hepatoxotoxicity was assessed by histologic examination and by estimations of serum glutamic pyruvic transaminase (SGPT), cytochrome P-450, and glutathione. Immediately after or 24 hours after anesthesia, the rats were sacrificed by cervical dislocation. Liver slices 1–2 mm thick were fixed in 10 per cent buffered formalin, pH 7.0, dehydrated in graded-strength ethanol, cleared in xylol, and embedded in paraffin. Sections 8 µm thick were stained with hematoxylin and eosin, and necrosis was evaluated by light microscopy. Histologic grading of the hepatic lesions was performed by a pathologist who was unaware of the treatment received by the rats. The grading was as follows:

0  No lesion present
0½+ Individual necrotic cells seen at the first cell layer away from the central vein, and hyaline degeneration present
1+ Necrotic cells extruding two or three cell layers away from the central vein
2+ Necrotic cells extruding three to six cell layers away from the central vein, but limited in peripheral distribution around the central vein
3+ The same as 2+, but with necrosis extending from one central vein to another
4+ More severe than 3+, with extensive centrilobular necrosis throughout the section

Samples of blood from groups of rats treated with either T₃ (10, 5, or 1 mg/kg/day) or vehicle (six to ten in each group: total number = 90 rats) were collected for determination of glutamic pyruvic transaminase (SGPT) by a quantitative colorimetric method§ before exposure to halothane, and immediately after and 24 hours after exposure to 1 per cent halothane in oxygen, 21 per cent.

§§ Ohio 200 Oxygen Monitor, Ohio Medical Products, 3030 Airco Drive, Madison, Wisconsin 53701.
† † Harlan Industries, Inc, Box 29176, Indianapolis, Indiana.
Fig. 1. Centrilobular necrosis in the liver from a triiodothyronine-treated rat (5 mg/kg, orally, 6 days) anesthetized with halothane, 1 per cent, in oxygen, 21 per cent, for two hours.

number = 24) were pretreated with Tween 80, and cytochrome P-450 estimations were then carried out before and immediately and 24 hours after anesthesia with halothane at the same oxygen concentration. Glutathione (GSH) was measured by the method of Richardson and Murphy,9 and cytochrome P-450 estimations were carried out by the method of Omura and Sato.10 In addition, the rats were weighed daily throughout the pretreatment period, and plasma T₃ concentrations were measured in groups of the rats that had received T₃.

Another series of experiments was carried out as described above except that six T₃-pretreated rats and six control rats treated with Tween 80 alone were anesthetized with enflurane, 1.8 per cent, in oxygen, 21 per cent, for two hours, or with pentobarbital, 40 mg/kg, ip, at an oxygen concentration of 21 per cent. Further control groups of rats (six in each group) received the same oxygen concentration, 21 per cent, without enflurane or pentobarbital. The concentration of enflurane was determined by gas chromatography.

The results were analyzed using a Student t test for unpaired data or the Wilcoxon nonpaired rank sum test where this was more appropriate, P < 0.05 being taken as the minimal level of significance.

Results

The plasma T₃ concentrations in T₃-pretreated rats ranged from 863 to >30,000 ng/100 ml (table 1). The T₃-treated rats gained weight more slowly than the control rats during the pretreatment period (table 2), because of their hyperthyroid state.

Centrilobular necrosis occurred in the livers of all rats that had received T₃ (1, 5, or 10 mg/kg) for six days and then had been anesthetized with halothane, 1 per cent, at an oxygen concentration of 21 per cent on the seventh day. The necrosis was variable in extent and severity, ranging from discernible hyaline droplet degeneration and vacuolation in a few, but not all, parenchymal cells immediately adjacent to central veins to extensive confluent lytic necrosis in the central regions with marked round-cell infiltration (fig. 1). Necrosis was not observed in the perportal region.

Histologic abnormalities were not found in the livers
of control rats that had received pretreatment with Tween 80 and then had been anesthetized with halothane or had received T₃ for six days but had not been exposed to halothane. There was no significant change in the histologic severity of the lesion (table 3) with increasing doses of T₃ when assessment was carried out immediately after halothane administration. At T₃, 10 mg/kg, there was no significant difference between the extents of the lesions immediately after halothane anesthesia and 24 hours after halothane administration. However, at the lower doses of T₃, the lesion had lessened significantly at 24 hours when compared with lesions examined immediately after exposure to halothane. In addition, 24 hours after exposure to halothane, there was a significant dose response, with worsening of the lesion with increasing doses of T₃. The lesion produced by pretreatment with orally administered T₃, 5 mg/kg, was significantly worse than that produced by 1 mg/kg, 24 hours after exposure to halothane, and the lesion produced by 10 mg/kg was significantly worse than that produced by 5 mg/kg. The lesion produced by T₃, 1 mg/kg, sc, was significantly worse than the lesion produced by the same dose given orally.

**Table 3.** Histologic Grading* of the Hepatic Lesions before, Immediately after, and 24 hours after Halothane Anesthesia† Following Six Days of Pretreatment with Orally Administered T₃

<table>
<thead>
<tr>
<th>Dose of T₃ mg/kg/day</th>
<th>Number of Rats</th>
<th>Pre-halothane</th>
<th>Post-halothane</th>
<th>24 Hours Post-halothane</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>15</td>
<td>0</td>
<td>2+ to 4+</td>
<td>1+ to 4+</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0</td>
<td>2+ to 3+</td>
<td>1+ to 2+</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>0</td>
<td>1+ to 3+</td>
<td>0 to ½+</td>
</tr>
</tbody>
</table>

* For explanation of grading, see text.
† Halothane, 1 per cent, in 21 per cent oxygen for two hours.

**Table 4.** Effects of Pretreatment with Triiodothyronine (T₃) and Phenobarbital and Various Oxygen Concentrations on Halothane-induced Hepatic Damage 24 Hours after Halothane Exposure

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Number of Rats</th>
<th>Extent of Histologic Damage at 14 Per Cent O₂</th>
<th>21 Per Cent O₂</th>
<th>99 Per Cent O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₃, 1 mg/kg, sc, 5 days</td>
<td>6</td>
<td>2+ to 4+</td>
<td>2+ to 3+</td>
<td>1+ to 2+</td>
</tr>
<tr>
<td>Tween 80 control</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T₃, 1 mg/kg, sc, 5 days, and phenobarbital, 75 mg/kg, per day, 3 days</td>
<td>5</td>
<td>2+ to 4+</td>
<td>2+ to 3+</td>
<td>1+ to 3+</td>
</tr>
<tr>
<td>Tween 80 control Phenobarbital, 75 mg/kg per day, 3 days</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tween 80 control</td>
<td>5</td>
<td>1+ to 3+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 2.** Serum glutamic pyruvic transaminase (SGPT) concentrations after various doses of triiodothyronine (T₃) (1, 5, and 10 mg/kg, orally, 6 days), before and immediately after and 24 hours after exposure to halothane in 21 per cent oxygen. Numbers of rats studied were: T₃, 10 mg/kg = 7; T₃, 5 mg/kg = 7; T₃, 1 mg/kg = 6; Tween 80 controls = 10.

Centrilobular necrosis occurred in the T₃-pretreated rats (1 mg/kg, sc, for five days) anesthetized with halothane in oxygen 14, 21, and 99 per cent (table 4). The lesion produced by T₃ and halothane was significantly less severe when oxygen, 99 per cent, rather than 21 per cent, was administered, suggesting that increasing oxygen concentrations protected against it. Pretreatment with phenobarbital alone (75 mg/kg/day, ip, for three days) resulted in hepatic centrilobular necrosis only when the animals were anesthetized in a hypoxic environment, in keeping with the models previously described. The addition of phenobarbital did not worsen significantly the lesion produced by halothane in the T₃-pretreated animals exposed to oxygen, 14, 21, and 99 per cent (table 4).
Fig. 3. Effects of pretreatment with triiodothyronine (T₃, 1 mg/kg, sc, 5 days) and halothane anesthesia (1 per cent in 21 per cent oxygen for 2 hr) on hepatic glutathione (GSH), mg/g liver. Number of rats studied in each group = 5.

The lesion produced by T₃ administration and halothane, 1 per cent, in oxygen, 14 per cent, was significantly worse than the lesion produced by phenobarbital pretreatment and halothane, 1 per cent, in oxygen, 14 per cent.

Levels of SGPT were increased significantly in the T₃-treated rats immediately after anesthesia with halothane in oxygen, 21 per cent (fig. 2). Pretreatment with Tween 80 alone did not increase SGPT values before or immediately after or 24 hours after exposure to halothane. However, there was a significant increase in the SGPT levels in the T₃-pretreated rats immediately following anesthesia, but by 24 hours, SGPT had returned to preanesthetic control values.

There was a significant decrease in glutathione (GSH) values 24 hours after T₃ administration, but no further decrease with continued T₃ pretreatment or with the administration of halothane in oxygen, 21 per cent (figs. 3 and 4).

Cytochrome P-450 was significantly decreased by T₃ pretreatment (table 5), and halothane anesthesia caused a further significant decrease. There was no such decrease in control rats exposed to halothane. However, there was no significant difference in cytochrome P-450 values obtained before anesthesia and 24 hours after halothane anesthesia in T₃-pretreated rats, indicating that cytochrome P-450 had returned to the lower preanesthesia levels at 24 hours in the pretreated rat.

No evidence of hepatocellular damage as assessed by histologic grading or SGPT levels was found in the rats anesthetized with enflurane or pentobarbital.

Discussion

Evidence from previous animal experiments suggests that the mechanism of halothane hepatotoxicity involves biotransformation to reactive metabolites, particularly through a reductive pathway¹,²,¹¹ that covalently bind to hepatic macromolecules. Our study clearly demonstrates that T₃ administration in doses sufficient to produce clinical and biochemical evidence of hyperthyroidism causes halothane-induced hepatic necrosis to occur at normal oxygen concentrations,
and that hyperoxia does not prevent the lesion. That we could not produce the lesion with enflurane or pentobarbital anesthesia suggests that it was not anes-
thesia per se that was responsible for the production of hepatic damage in our experimental model, but rather, that halothane played a key role in producing hepatotoxicity.

There was a significant decrease in glutathione levels 24 hours after T3 administration, but no further decrease with continued T3 pretreatment or with the administration of halothane. The mechanism of acet-
aminophen hepatotoxicity and the role of glutathione have recently been defined in a series of experiments by Brodie and co-workers.12-15 They showed that the covalent binding of reactive intermediates is responsible for the hepatotoxicity of acetaminophen over-
dosage, and it has been suggested the production of halothane metabolites that covalently bind to hepatic macromolecules might be responsible for halothane hepatotoxicity.14-13 The administration of acetaminophen causes a dose-dependent depletion of hepatic glutathione in mice.14 In addition, covalent binding of the acetaminophen metabolites to hepatic macromolecules does not occur until the availability of glutathione is exhausted through conjugation with the metabolite. Glutathione can thus protect the liver from the toxic effects of acetaminophen and other drugs such as the anesthetic agent chloroform.16 The exact mechanism has not been defined, but it appears that glutathione by combining with the reactive inter-
mediates decreases covalent binding of these metabolites to hepatic macromolecules. Brown and co-workers investigated the production of chloroform toxicity16 and demonstrated that enzyme induction caused a large increase in in-vitro covalent binding of metabolites of 14C chloroform to hepatic microsomal protein, and that this binding could be prevented by glutathione. Brown showed that two hours of chloroform, 0.5 per cent, decreased hepatic glutathione content by 70 per cent, but there was no change in glutathione concentrations with halothane anesthesia even in enzyme-induced rats. We found no change in hepatic glutathione content with halothane anesthesia in our model, but T3 itself reduced glutathione content, and this may have important implications in the production of hepatotoxicity in our experimental model.

We also have demonstrated that T3 pretreatment decreases cytochrome P-450, and that a further de-
crease occurs with halothane anesthesia, but no change in cytochrome P-450 with halothane anesthesia oc-
curs in the control rats. VanDyke has shown that halothane biotransformation occurs in the microsomal fraction of the hepatic cell by the mixed function oxi-

dase system17 and is inducible by phenobarbital.18 Cytochrome P-450 is thus believed to play a key role in the biotransformation of halothane, and may be involved in the formation of toxic metabolites. In the animal models of halothane hepatotoxicity in which enzyme induction has been used, increase in cytochrome P-450 appears to play an important role. However, in our model, total cytochrome P-450 is decreased by T3 administration. This confirms the work of other investigators, who have also shown that changes in thyroid state can affect the mixed-function oxidase system19; it has been demonstrated that cytochrome P-450 is decreased by thyroxine treatment in male rats, but not in female rats.20 However, it should be recognized that a hitherto undetermined subfraction of the microsomal enzyme system may be increased.

We have shown that the administration of thyroid hormone causes hepatic centrilobular necrosis to occur in rats anesthetized by halothane at an ambient oxygen concentration. Although the mechanism is unknown, the possibility exists that excess T3 induces an intracellular hypoxia, causing halothane to under-
go reductive biotransformation to reactive metabo-
lites, which produce hepatic damage.

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Table 5. Cytochrome P-450 Estimations (Mean ± SEM) in Rats Pretreated with Triiodothyronine (T3), 1 mg/kg, Five Days before and Immediately and 24 Hours after Anesthesia with Halothane, 1 Per Cent, in Oxygen, 21 Per Cent

<table>
<thead>
<tr>
<th></th>
<th>T3 6 Rats</th>
<th>Control 6 Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>No anesthesia</td>
<td>0.41 ± 0.01</td>
<td>0.97 ± 0.04*</td>
</tr>
<tr>
<td>Immediately after halothane</td>
<td>0.27 ± 0.04*</td>
<td>0.95 ± 0.06*</td>
</tr>
<tr>
<td>No anesthesia</td>
<td>0.40 ± 0.03</td>
<td>0.89 ± 0.04*</td>
</tr>
<tr>
<td>24 hours after halothane</td>
<td>0.40 ± 0.04</td>
<td>0.92 ± 0.06</td>
</tr>
</tbody>
</table>

* P < 0.05.


