Hepatic Necrosis Produced by Repeated Administration of Halothane to Guinea Pigs

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It was the purpose of this experiment to produce hepatic lesions in the guinea pig by repeated administration of halothane as an anesthetic agent. Although no clinical chemistry or hematologic findings suggested hepatic disease, seven of 50 guinea pigs anesthetized one to five times with halothane had hepatic lesions. The lesions were characterized by a focal, diffuse hepatic necrosis infiltrated with mononuclear cells. (Key words: Hepatic necrosis; Guinea pig (Cavia porcellus); Halothane; Liver.)

HALOTHANE is commonly used for general anesthesia in man and animals. In a small proportion of human subjects, halothane anesthesia is followed by hepatic necrosis.1-4 Inability to reproduce a similar syndrome in laboratory animals has made it difficult to determine the pathogenesis of this condition.5-9

It was the purpose of this experiment to produce hepatic lesions in the guinea pig by repeated anesthetization with halothane.

Materials and Methods

Seventy female Dunkin-Hartley guinea pigs (Cavia porcellus), each weighing approximately 500 g, were randomly divided into seven groups of ten animals each (table 1). The two groups which were used as controls were not anesthetized, but were placed in a clear plastic chamber for an hour, during which time control group I breathed room air and control group II breathed 100 per cent oxygen administered at a flow rate of 4 l/min. Five of the guinea pigs from the first group were killed at the end of the hour and five were killed 11 weeks later. Five of the animals from the second control group were killed immediately after the oxygen treatment, and five were killed a week later.

The five experimental groups were anesthetized one to five times for an hour in the clear plastic chamber. The anesthetic (1 per cent halothane † in oxygen, administered at a flow rate of 4 l/min § in a flow-through system) was given at two-week intervals. Five guinea pigs from each group were killed by decapitation immediately after the last period of anesthesia; the remaining five were killed a week later.

All guinea pigs were killed by decapitation. Blood samples, both clotted and uncotted, were collected immediately after death. A necropsy of each animal was performed, and representative tissues were fixed in a 10 per cent solution of neutral buffered formalin. Selected tissues for paraffin sectioning were taken from the liver and stained with hematoxylin and eosin.

The uncotted blood samples were used for leuкоyte and differential counts. Clotted blood samples were centrifuged at 3,000 rpm for 10 minutes and the decanted serum was used for laboratory studies. The serum glutamic pyruvic transaminase (SCPT) and serum

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HEPATIC NECROSIS IN GUINEA PIGS

Table 1. Designation of Groups According to Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Total Number of Animals</th>
<th>Number of Times Anesthetized</th>
<th>Number Killed Immediately after Final Anesthesia</th>
<th>Number Killed One Week after Final Anesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I</td>
<td>None</td>
<td>10</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Control II</td>
<td>Oxygen</td>
<td>10†</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Experimental I</td>
<td>Halothane</td>
<td>10</td>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Experimental II</td>
<td>Halothane</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Experimental III</td>
<td>Halothane</td>
<td>10</td>
<td>3</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Experimental IV</td>
<td>Halothane</td>
<td>10</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Experimental V</td>
<td>Halothane</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

* Five animals were killed at the termination of treatment and five, 11 weeks later.
† Five animals were killed after one hour of oxygen and five, one week later.

Glutamic oxalacetic transaminase (SGOT) levels were measured by the Reitman-Frankel method. Total lactate dehydrogenase (LDH) was determined by the Babson-Phillips method and the isoenzyme fractions were then separated by electrophoresis on cellulose acetate for an hour at 250 volts; tris-barbital at pH 8.6 was used as the buffer. After the electrophoretic separation was completed, the isoenzyme pattern was developed by staining with a combination of tetracium dye, phenazine monosulfate, and nicotinamide adenine diphosphate for 30 minutes at 37°C. The cellulose acetate strips were washed in tap water, dried at room temperature, and then scanned with a Densicord Model 547 (Photovolt Corporation; New York, N. Y.) for quantification of the isoenzyme fractions.

Results

There were no signs of adverse effects due to halothane either during or after anesthesia. The 1 per cent halothane kept the guinea pig in a plane of light surgical anesthesia. The respiratory rates decreased slightly, but not significantly, and body temperatures remained constant during anesthesia. The guinea pigs regained their righting reflexes within 9 minutes after removal from the anesthesia chamber. There were no significant differences between the lengths of recovery times in any of the groups.

The total leukocyte counts, differential counts, SGPT, SGOT, and total LDH levels showed no significant differences between any of the groups, and none of the individual SGPT values was greater than 50 Reitman-Frankel units/ml. Although several animals had SGOT levels above 50 Reitman-Frankel units/ml, this was not a consistent finding. The total LDH levels and the mean response of the LDH isoenzymes were not indicative of severe hepatocellular injury. No eosinophilia or lymphocytosis was found in any group.

No gross lesions were observed at autopsy, in the liver or elsewhere; but focal hepatic lesions visible by light microscopy were seen in seven of the 50 anesthetized guinea pigs: one animal in each of the first three experimental groups, and two animals in each of the last two (table 2). None of the animals in either control group had comparable lesions. The extent of parenchymal damage in the affected animals ranged from less than 25 per cent of the lobule in experimental groups I, II, and III to approximately 50 per cent in experimental groups IV and V.

Table 2. Lesions Produced by Repeated Halothane Anesthetics in Guinea Pigs

<table>
<thead>
<tr>
<th>Number of Times Anesthetized</th>
<th>Number of Animals Affected</th>
<th>Severity of Lesion*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>+</td>
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<tr>
<td>3</td>
<td>1</td>
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<tr>
<td>4</td>
<td>2</td>
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</tr>
<tr>
<td>5</td>
<td>2</td>
<td>++</td>
</tr>
</tbody>
</table>

* + = Hepatic necrosis of 25 per cent or less of the lobular parenchyma.
++ = Hepatic necrosis of 25-50 percent of the lobular parenchyma and mononuclear infiltration.
Fig. 1. The earliest type of lesion was seen as degenerating hepatic cells radiating from a central vein. Hematoxylin and eosin, x925.

The lesions seen in the first three groups were those of early hepatic necrosis (fig. 1). The degenerated hepatic cells were swollen and had a homogeneous basophilic cytoplasm. Nuclei were shrunken, irregularly shaped, and without nucleoli. These lesions radiated out from the central vein along the hepatic cords.

A second type of lesion, seen in the animals in experimental groups IV and V, was characterized by degenerating hepatocytes, mononuclear cells, and cellular debris; these lesions were most numerous around the central vein, and extended into the lobule. In some areas it appeared that hepatocytes had “dropped out” and been replaced by mononuclear cells and cellular debris.

Larger areas of necrosis were usually seen in the midzonal region (fig. 2). These lesions were characterized by a central core of necrotic hepatocytes with a homogeneous basophilic cytoplasm and pyknotic or absent nuclei. The necrotic area was surrounded by degenerating hepatocytes and an infiltrate composed of mononuclear cells.

Areas of pericholangiolitis were seen (fig. 3); these were characterized by infiltrates of mononuclear cells and accumulations of erythrocytes and cellular debris. Necrotic hepatocytes were found in large areas surrounding the hepatic triads, and the hepatic cells at the periphery of the lesion showed early degenerative changes. The entire lesion was infiltrated with mononuclear cells.
Discussion

Hepatocellular injury due to chemical agents can be divided into two major types: 1) toxic reactions due to compounds such as carbon tetrachloride, which are dose-related and appear in all individuals when sufficient amounts are administered; and 2) hypersensitivity reactions, which are not strictly dose-related and occur in only a small proportion of exposed individuals.\(^4\)\(^,\)\(^12\)\(^,\)\(^13\) Halothane hepatitis in man is probably of the second type, although some investigators believe it may be the result of a toxic reaction.\(^4\)\(^,\)\(^14\)

Focal diffuse hepatitis was seen in seven guinea pigs anesthetized with halothane. Evidence of hepatocellular injury includes swollen cells showing fatty degeneration and shrunken cells with a homogeneous basophilic cytoplasm; the nuclei of these degenerated cells were either pyknotic or absent. The intralobular inflammatory response consisted primarily of small mononuclear cells in and around the degenerating hepatocytes.

The reason for the absence of serum enzyme changes in the guinea pigs with hepatic necrosis is not clear. It may be that those animals killed immediately after anesthesia did not have sufficient time to develop serum enzyme changes, since the injury was initiated only an hour prior to death. It is also possible that the clinical chemistry methods used may not have been sensitive to the disease state observed. The enzyme changes due to hepatic damage are species-specific\(^12\); how-
ever, the methods used have been determined to be valid for more severe hepatic pathology in the guinea pig.

Although fatty changes have been produced in the livers of mice following oral administration of halothane, this is the first time hepatic necrosis has been seen in a species other than man following halothane anesthesia. The failure to produce hepatic necrosis by the use of halothane anesthesia in other laboratory animals may have been due to the relatively short duration of the experiments or to the fact that the other animals used may be less susceptible than the guinea pig to hepatic injury induced by halogenated hydrocarbons. Apparently, repeated exposures are needed to produce hepatic necrosis associated with a mononuclear cell response, since lesions of this type were not observed until 48 days after the initial period of anesthesia.

The production of hepatic necrosis in the guinea pig would suggest that this animal would make an excellent model for the study of halothane-induced hepatic injury.

References


Neonatology

**BODY WATER AT BIRTH** Total body water (antipyrine space) and extracellular water (corrected bromide space) were measured within 19 hours of birth in 16 infants delivered vaginally and 17 infants delivered by cesarean section. The infants delivered by cesarean section had a significant increase in total body water, 77 ml/kg, mainly in intracellular water. In the first eight hours of life, the infants delivered vaginally had significant decreases in total body water, mainly intracellular, together with slight increases in extracellular water. *(Cassady, G.: Effect of Cesarean Section on Neonatal Body Water Spaces, N. Eng. J. Med 285: 887–891, 1971.)*