Anesthesiology
61:544–550, 1984

The Involvement of Endotoxin in Halothane-associated Liver Injury

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Since endotoxin, lipopolysaccharides (LPS), have been implicated as a causative factor in the development of hepatic necrosis in rats exposed to hepatotoxic levels of several chemical agents, the role of LPS in the halothane–hypoxia (HH) model of hepatic damage in male Sprague–Dawley rats was investigated. When injected intravenously immediately after halothane anesthesia, a subnecrotic dose of LPS (0.5 mg/kg; *Escherichia coli* 026:B6) was found to markedly potentiate HH-induced hepatic necrosis. Pretreatment of the animals with the antiendotoxin agent, lactulose, prior to exposure to halothane reduced the hepatic damage normally seen from HH. A possible mechanism of LPS-induced potentiation was indicated by changes in hepatic calcium levels at 24 h after treatment. Endogenous LPS may play a role in HH-induced hepatic necrosis, and the mechanism of LPS-induced potentiation may be due to an LPS-related membrane dysfunction. (Key words: Anesthetics, volatile: enflurane; halothane. Ions: calcium. Liver: hepatotoxicity. Toxicity: endotoxin; hepatic; metabolites.

HALOTHANE has been implicated in hepatic damage in humans that can progress to a fulminant and fatal hepatitis.[1] A current hypothesis is that binding of reactive metabolites of halothane to subcellular macromolecules causes transient minor damage, whereas production of antibodies to the halothane-altered hepatocyte membranes leads to the fulminant form of the disease.[2–4] A model (halothane–hypoxia; HH) consistently demonstrating hepatic damage in rats has been developed by induction of hepatic microsomal enzymes with phenobarbital followed by 1% halothane with reduced oxygen tension ($F_{I_{O_2}} = 0.14$) for 2 h.[5,6]

Endotoxins, lipopolysaccharides (LPS), originating from the cell walls of the gram-negative bacterial flora of the lower intestine are potential hepatic and systemic toxins.[7] The reticuloendothelial system of the liver is the major means of detoxification of LPS normally arriving from the lower gut via the portal vein.[7] LPS have been shown to both potentiate and be involved intrinsically in the acute hepatotoxic actions of CCl_4[7,8] and galactosamine[9,10] in rats.

The hepatic injury induced by the HH model and associated models produces only a mild transient hepatic necrosis.[2] In addition, questions have been raised as to whether or not halothane produces a chemical insult to the liver that is truly responsible for the hepatic necrosis.[11]

Thus, the role of LPS in anesthetic-associated liver injury in rats was examined by determining if exogenously introduced LPS would potentiate HH-induced hepatic necrosis and if administration of an antiendotoxin agent would ameliorate the liver injury associated with the HH model.

Materials and Methods

Halothane was obtained from Ayerst Laboratories (New York, New York) and enflurane from Ohio Medical Products (Madison, Wisconsin). Reference 1% halothane and 2% enflurane precision gas mixtures (±0.01%) diluted in nitrogen were purchased from Matheson Gases (Cucamonga, California). Sodium phenobarbital was purchased from the Mallinckrodt Company (Paris, Kentucky). Albumin protein standard stock solution, lipopolysaccharides; *E. coli* 026–B6 trichloroacetic acid extracted (lot #111F-4023), and serum glutamate pyruvate transaminase kits all were obtained from Sigma Chemical Company (St. Louis, Missouri). Lactulose was obtained as the commercial preparation of 66% lactulose syrup, Chronulac®, from Merrell Dox Pharmaceuticals (Cincinnati, Ohio). Shellac-free Pelikan Fount India Ink® (Pelikan AG, Hanover, West Germany) was suspended in Knox® Unflavored Gelatin (Englewood Cliffs, New Jersey). Calcium standards were prepared from reagent grade calcium chloride supplied by the J. T. Baker Chemical Company (Phillipsburgh, New Jersey). Lanthanum oxide was obtained from the Eastman Kodak Company (Rochester, New York). All other chemicals were of reagent grade or better.

ANIMALS

Male Sprague–Dawley rats (250–400 g) were obtained from Hilltop Laboratories (Hilltop, Pennsylvania). The rats were housed in pairs in stainless steel cages in an isolated room with the independent air supply. A 12-h light/dark cycle and a temperature of 22° C were maintained. The rats were allowed standard laboratory rat chow and water *ad libitum*.

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Received from the University of Arizona, Health Sciences Center, Tucson, Arizona 85724. Accepted for publication May 2, 1984. Supported by NIH Grant AM 16715. Preliminary reports of this research appeared as abstracts in Pharmacologist 24:193, 1982; ANESTHESIOLOGY 57:A220, 1982; Toxicologist 3:462, 1983.
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Animal Pretreatment

As previously described for our HH model,[5,6] the hepatic cytochrome P-450 drug-metabolizing enzymes were induced by treating the rats with a single intraperitoneal (ip) injection of 100 mg/kg sodium phenobarbital followed by 5 days of maintenance on drinking water containing 1 mg/ml sodium phenobarbital. The rats then were allowed 24 h on tap water before utilization in experiments.

Animals used in the lactulose pretreatment experiments had their hepatic enzymes induced via three daily ip injections of 80 mg/kg sodium phenobarbital beginning 4 days before halothane anesthesia. This protocol was used to avoid simultaneous administration of two agents in the drinking water.

Inhalation Exposure Conditions

Animals were exposed to 1% v/v halothane or 2% v/v enflurane in a hypoxic (FI_{O_2} = 0.14) or normoxic (FI_{O_2} = 0.21) environment for 2 h in a 180 l plexiglass chamber, as previously described.[6]

Lipopolysaccharide Administrations

E. coli 026-B6 endotoxin was dissolved in 0.9% sterile saline at concentrations to allow administration of 1 ml/kg either ip or iv. Intravenous dosings were all done via the tail vein.

Lactulose Administration

Commercial 66% lactulose syrup was diluted with tap water to a 10% solution and substituted for drinking water for 7 days before experimentation, a procedure shown to reduce stool *p*H and alter gut flora.[12] Daily consumption of the 10% lactulose solution was monitored using volumetrically calibrated water bottles.

To insure that the lactulose treatment did not alter the induction of the hepatic biotransformation enzymes by phenobarbital, rats were killed following the lactulose plus phenobarbital and phenobarbital-only treatments and their hepatic cytochrome P-450 content determined.[6]

Evaluation of Hepatotoxicity

Following cervical dislocation, blood was drawn from the inferior vena cava into heparinized syringes. Plasma was obtained by centrifugation and stored at 4° C. Glutamate–pyruvate transaminase (GPT) levels were determined on the plasma samples (Sigma Technical Bulletin No. 55—UV) and expressed as Wroblewski–LaDue (W-L) units.

After rats were killed, part of the liver was frozen while other liver sections were fixed in a solution of 10% phosphate-buffered formalin and processed into 7-μm-thick slices that were stained with hematoxylin and eosin (H and E).

Previously reported means of evaluating hepatic necrosis caused by halothane are highly subjective with the assigning of numbers based on overall histologic appearance.[6] Also, vacuolization often is identified as necrosis by these methods. Therefore, an attempt has been made to quantitatively evaluate actual necrosis, *i.e.*, cell death, by observing the infiltration of macrophages (MP), which normally migrate to an area of inflammation in order to phagocytize cellular debris.[13] Each hepatic histologic sample was observed through a 10 × 10 grid at 40 ×, which is an area of 4 mm^2, and the squares demonstrating infiltrations of MP were totaled. This was done for six randomly chosen fields on each slide, with the average value being reported as the mean per cent incidence of MP infiltration.

Carbon Clearance by the Reticuloendothelial System

The reticuloendothelial systems of HH-exposed rats were evaluated for their ability to clear small carbon particles from the blood using a modification of the method of Biozzi *et al.*[14] Shellac-free Pelikan Fount India Ink® was centrifuged at 12,000 × *g* for 15 min to remove the larger carbon particles. Aliquots of the supernatant then were lyophilized to estimate the carbon content. The ink in the supernatant fraction then was diluted with water with the addition of sufficient gelatin to produce a colloidal suspension of 40 mg/ml carbon in 2% gelatin. Rats dosed with up to 160 mg/kg iv of this solution showed no adverse reactions. At 2 h after HH, the rats were dosed with the colloidal carbon suspension (80 mg/kg, iv) via the tail vein. Phenobarbital-pretreated animals not exposed to halothane served as controls. Blood samples were collected from the retroorbital plexus using a 20-μl glass capillary pipets that had been prerinsed with heparin solution (1,000 units/ml) and dried. Samples were taken just prior to infusion of the colloidal carbon and then every 5 min for 25 min.

The collected samples were dissolved in 2 ml of 0.1% Na_2CO_3 and then sonicated. The disappearance of the carbon from the blood was measured by absorbance of the samples at 600 nm using the preinjection samples as the blank. The half-lives of the colloidal carbon in the blood were used to compare the effect of HH on the clearing ability of the reticuloendothelial system.

Hepatic Tissue Calcium Levels

All glassware used was soaked in 6 N HCl for 3 days before use and then rinsed with double-distilled water. Two grams of frozen liver tissue were homogenized in

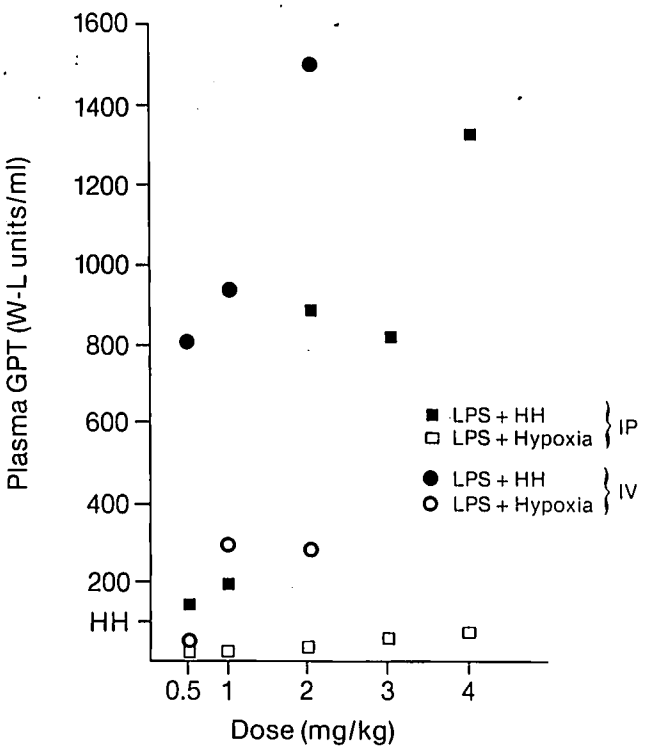

FIG. 1. Dose–response potentiation by LPS of liver injury in the HH model. Twenty-four hour GPT values resulting from the administration of LPS ip immediately before hypoxia (FI_{O_2} = 0.14) (□) or HH (■). Twenty-four-hour GPT levels resulting from the administration of LPS iv immediately before hypoxia (FI_{O_2} = 0.14) (○) or HH (●). Error bars not shown for clarity (n = 3). Standard deviations were 20–100% in all cases.

4 ml of double-distilled water. Duplicate 1 ml aliquots of the homogenates were placed into preweighed beakers and lyophilized to determine the dry weight of the homogenized tissue. The dried samples were dissolved in 5 ml of nitric acid and then oxidized with 30% hydrogen peroxide (5 ml). These dissolved samples were evaporated to dryness at 150–200° C. Ten milliliters of 0.5% lanthanum trichloride in 0.1 N HCl were added to each beaker and the solution transferred to plastic tubes. Any particulate matter was removed by centrifugation at 1,000 × *g* for 15 min, and the supernatant was decanted carefully into another plastic tube. These solutions were analyzed for calcium content by atomic absorption. Standards of 1 to 5 ppm calcium were prepared by dissolving calcium chloride in a 0.5% lanthanum chloride solution.

TABLE 1. Potentiation of HH-Initiated Hepatic Injury by LPS Administration at Various Times after HH Exposure

Time† (h)	Plasma GPT*	
	HH‡	HH + LPS§
24	104 ± 70 (9)	284 ± 192¶ (9)
48	90 ± 69 (9)	235 ± 135¶ (5)
72	28 ± 1 (5)	196 ± 139¶ (5)
96	22 ± 4 (5)	24 ± 12 (4)

* W-L units/ml; ( ) = n.
† Time after HH exposure; LPS administration 24 h before time point.
‡ Phenobarbital induction, 1% halothane, FI_{O_2} = 0.14, 2 h.
§ 0.5 mg/kg, iv.
¶ $P < 0.01$ *versus* HH values at same time point.

EXPERIMENTAL PROTOCOL

To best determine the dose and route of administration of LPS for potentiation of the HH model with minimal toxicity expressed by the LPS alone, rats received a range of doses of LPS either ip or iv immediately prior to hypoxia (FI_{O_2} = 0.14) or HH (n = 3 for each dose and exposure condition). At this point in the study it was decided that in order to avoid possible alterations by LPS in the development of the HH lesion, LPS, henceforth would be administered after HH. Thus, any potentiation would occur after the initial damage from HH.

A time course of the hepatotoxic effects of hypoxia and LPS, HH, and HH + LPS was followed up to 72 h after treatment to determine the time of maximal potentiation by LPS of the HH model (n = 4–9 for each time point). LPS also was administered at 0, 24, 48, and 72 h after HH into animals (n = 4–9 at each time point) in order to evaluate how long after HH exposure LPS still would potentiate the lesion.

The possibility that endogenous LPS is involved in the development of necrosis in the HH model was tested by pretreating the rats with lactulose, a proven antiendotoxin agent[12] and observing changes in the development of the hepatic lesion at 24 h after HH exposure. This experiment was performed in duplicate (n = 4).

Indicators of two mechanisms that possibly could be increasing the liver's susceptibility to LPS-induced damage after HH also were investigated. The functional ability of the reticuloendothelial system, which normally removes and detoxifies LPS, was evaluated by measuring the clearance rate of colloidal carbon from the blood of phenobarbital-induced and HH-exposed animals (n = 4). Because of the work of Fritz and Keppler,[15] which demonstrated increased calcium influx into the hepatocytes of female mice within 4 h of the administration of 0.5 mg/kg LPS, and since the influx of calcium ions into hepatocytes is considered a mechanism of cellular dysfunction and ultimately cell death,[16] the effect of our treatment regimens on the concentration of hepatic calcium was investigated.

Finally, to demonstrate that the potentiation of the toxicity of the HH model by LPS is not due to anesthesia

TABLE 2. Potentiation with LPS and Reduction by Lactulose Pretreatment of HH-Induced Hepatic Damage

Treatment	n	Plasma GPT* (W-L Units/ml)	Hepatic Per cent MP Infiltration†
Hypoxia‡	6	22 ± 4	0.5 ± 0.1
Hypoxia + LPS§	10	30 ± 9	2.9 ± 0.5
HH¶	11	103 ± 70	9.8 ± 3.8
HH + LPS	9	419 ± 300**	18.8 ± 4.0**
Lactulose†† + HH	8	31 ± 17**	4.4 ± 3.8**

* $\bar{x}$ ± SD, 24 h after treatment.
† Mean macrophage infiltrations of 6 random fields/histology slide ± SD, 24 h after treatment.
‡ Phenobarbital induction, FI_{O_2} = 0.14, 2 h.
§ 0.5 mg/kg, iv, immediately after exposure.
¶ Phenobarbital induction, 1% halothane, FI_{O_2} = 0.14, 2 h.
** $P < 0.05$ *versus* HH values.
†† Ten per cent in drinking water, 7 days before HH.

per se, phenobarbital-induced rats (n = 5) were exposed to FI_{O_2} = 0.14 for 2 h or to 2.0% enflurane for 2 h at FI_{O_2} = 0.14 or 0.21 and then were injected with 0.5 mg/kg LPS iv immediately after exposure.

Statistical Analysis

Since plasma GPT value have large standard deviations that increase with increasing means, the log GPT was used to evaluate the significance of these values. Comparison of GPT values from the administration of LPS at various times after HH (table 1) were by *a priori* contrasts within two-way analysis of variance. All other data were analyzed by one-way analysis of variance and the means compared by Student's *t* test with *P* values less than 0.05 considered significant.

Results

Potentiation of the HH Model with LPS

LPS proved to be a potent potentiator of the hepatic damage caused by exposure to halothane under conditions of the hypoxic model. Whether administered by an ip route or by an iv route, before or after exposure, LPS administration caused a several-fold increase in 24 h GPT over HH-only treated animals (fig. 1; tables 1 and 2).

When administered ip and followed by hypoxia, LPS caused an increase in 24 h GPT only at doses greater than 3 mg/kg, while the iv route led to increased 24-h GPT levels at doses of only 1 mg/kg (fig. 1). Potentiation of the HH-induced hepatic damage by LPS was evident at all doses and by both routes of administration. However, because of its fairly consistent potentiation of the HH model, while showing little or no damage in hypoxia controls, the 0.5 mg/kg LPS iv dose was chosen for subsequent experiments.

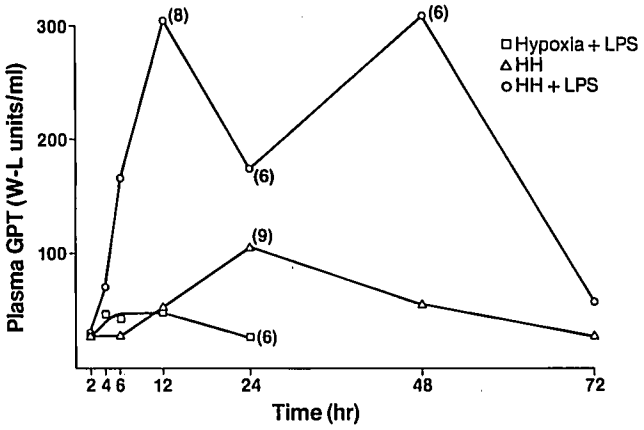

FIG. 2. Time course of the increase in GPT in HH (△), hypoxia (FI_{O_2} = 0.14) + LPS (□), and HH + LPS (○) treated rats. LPS injection (0.5 mg/kg, iv) immediately after exposure. Error bars not shown for sake of clarity. Standard deviations were 20–100% of values. n = 4 unless otherwise indicated ( ). Note: The 24 h HH + LPS value is not significantly different from 12 and 48 h values and has ranged from 300 to 800 W-L units/ml in other experiments. (Fig. 1, Tables 1 and 2).

Time Course of LPS-Induced Potentiation of the HH Model

In the hypoxia + LPS-treated animals, both GPT and per cent MP infiltration into the liver began to increase 2 h post treatment, leading to a very minor degree of injury with a maximum at 12 h (figs. 2 and 3). The chronology of the HH model followed a course similar to that previously reported,[6] with indices of necrosis not increasing until 6–12 h after exposure and peaking at

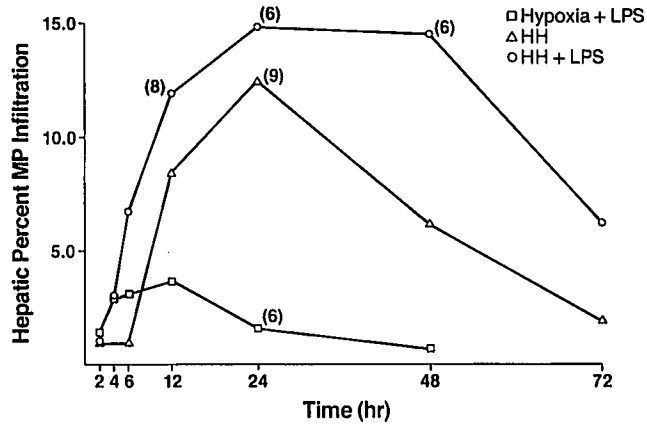

FIG. 3. Time course for the increase in hepatic per cent macrophage (MP) infiltration of HH (△), hypoxia (FI_{O_2} = 0.14) + LPS (□) and HH + LPS (○) treated rats. LPS injection (0.5 mg/kg, iv) immediately after exposure. Error bars are not shown for sake of clarity. Standard deviations were 10–50% of values. n = 4 unless otherwise indicated ( ). Note: the 24 h HH + LPS values have been as high as 19% in other experiments (Table 2).

TABLE 3. Effect of LPS and Lactulose Administration on Liver Calcium Levels after HH

Treatment	n	Liver Calcium* (ppm dry wt)	Plasma GPT* (W-L units)
Phenobarbital†	4	139 ± 7‡	23 ± 5
HH§	6	159 ± 11‡	119 ± 66‡
Hypoxia + LPS¶	6	180 ± 13‡	32 ± 11‡
HH + LPS	6	226 ± 47‡	414 ± 272‡
HH + Lactulose**	6	140 ± 5	30 ± 15

* $\bar{x}$ ± SD, 24 h after treatment.
† Phenobarbital induction only, 48 h after treatment.
‡ All groups differ by $P < 0.05$.
§ Phenobarbital induction, 1% halothane, $F_{I_{O_2}} = 0.14$, 2 h.
¶ $F_{I_{O_2}} = 0.14$, 2 h, 0.5 mg/kg LPS iv, immediately after.
** 10 per cent in drinking water, 7 days before.

24 h after, followed by a rapid resolution toward normal values. The GPT values for the HH + LPS-treated rats began to increase by 4 h after treatment with peak levels at 12 h. These values remained increased until 48 h, after which time the values decreased toward normal. Although large, the decrease in the HH + LPS GPT values at 24 h did not prove significantly different from the 12 and 48 h values. In fact, other experiments produced mean 24 h GPT values that ranged from 300–800 W-L units/ml in HH + LPS treated animals (tables 1 and 2; fig. 1). The hepatic per cent MP infiltration in the HH + LPS animals also began to increase rapidly by 4 h after treatment, peaked at 24 h after, and returned toward normal values after 48 h. Again, in other experiments the 24 h hepatic per cent MP infiltration values resulting from HH + LPS have been as high as 19% (table 2).

Temporal Relationship Between HH and Time of LPS Administration

LPS still could potentiate the hepatic damage caused by HH even when administered up to 48 h after the end of exposure (table 1). In fact, no differences were noted in the degree of LPS-induced potentiation between 0 time (end of exposure) and 48 h. By 72 h after exposure, this dose of LPS did not potentiate HH injury, nor did it produce injury.

Effect of Lactulose on HH-Induced Hepatic Damage

Rats maintained on 10% lactulose drinking water over a 7-day period consumed 6.3 ± 0.3 g lactulose · kg^{-1} · day^{-1}. This treatment regimen did not influence the ability of phenobarbital to induce the levels of hepatic microsomal cytochrome P-450. (lactulose treated:1.05 ± 0.35 nmol/mg protein, normal:1.09 ± 0.05 nmol/mg protein; n = 4 in each group).

Lactulose administrations for 7 days prior to HH dramatically reduced the hepatic damage produced in the HH model. There was a threefold decrease in the GPT levels and a twofold decrease in MP infiltration (table 2).

Carbon Clearance by the Reticuloendothelial System

When the clearance of the carbon particles from the blood was monitored after HH, no differences were observed. The calculated half-lives for the disappearance of the colloidal carbon was 10.8 ± 1.3 and 10.6 ± 1.3 min for the control and HH groups, respectively (n = 4 in each group).

Hepatic Calcium Levels after Treatment with LPS, HH, and HH + LPS

Significant increases in hepatic calcium levels were found at 24 h after HH (table 3), while the subnecrotic dose of LPS caused an increase in hepatic calcium to a level greater than that achieved by HH. The HH and LPS combination of treatments appears to increase hepatic calcium levels arithmatically, while causing a synergistic increase in 24 h GPT. The livers from rats pretreated with lactulose showed no increase in liver calcium after HH.

Enflurane and LPS

After anesthesia with 2% enflurane at $F_{I_{O_2}} = 0.14$ or 0.21 for 2 h followed by injection of 0.5 mg/kg LPS iv, the 24 h GPT levels were not increased above those observed in phenobarbital-treated rats exposed only to hypoxia and injected with LPS ($F_{I_{O_2}} = 0.14$ + LPS: 26 ± 6; enflurane, $F_{I_{O_2}} = 0.14$ + LPS: 28 ± 5; enflurane, $F_{I_{O_2}} = 0.21$ + LPS: 25 ± 5; n = 5 in all groups).

Discussion

Evidence accumulated over the last 40 yr in a variety of species has shown that initial damage to the liver by hepatotoxic agents such as CCl_4 and galactosamine greatly increases susceptibility to further hepatic necrosis and death from exogenously introduced LPS.[10,17,18] It also has been shown that endogenous LPS produced by enteric gram-negative bacteria may be responsible for the hepatic necrosis ultimately arising from these agents as well as from necrogenic diets.[7,9] The primary evidence for this link has been the reduction and even prevention of necrosis by either the elimination of enteric bacteria or the inactivation of LPS with antiendotoxins. In the case of dietary-induced hepatic necrosis, treatment with the nonabsorbed antibiotic neomycin prevented necrosis.[7] Pretreatment with nonabsorbed antibiotics,[19] the antiendotoxin polymyxin B,[8] or LPS-absorbing colestyr-

amine resins[20] greatly reduced hepatic damage normally caused by CCl_4. Galactosamine necrosis can be eliminated by pretreatment[12] or posttreatment[21] with lactulose or by performing a colectomy.[9]

LPS appears to play a similar role in the HH model for hepatotoxicity used here. A subnecrotic dose of exogenous LPS significantly potentiated the hepatotoxicity observed in rats exposed to HH, while a 7-day pretreatment with the antiendotoxic agent, lactulose greatly reduced HH-associated liver injury. LPS administration potentiated the HH lesion up to 48 h after HH exposure but not at 72 h after. This is consistent with the temporal evolution of the HH model in that the HH lesion is primarily resolved by 72 h and thus unresponsive to the dose of LPS. In the time course of lesion development, a combination of hypoxia and LPS caused minimal changes in the parameters of hepatic damage, while LPS potentiation of the HH model led to a more rapid onset and higher values over HH for both indices of liver necrosis.

Anesthesia *per se* would not be considered a factor in this LPS-induced potentiation, since administration of the same dose of LPS after enflurane anesthesia with or without hypoxia did not result in any evidence of hepatotoxicity. Thus, halothane itself is probably responsible for the increased susceptibility to LPS, perhaps resulting from the bioactivation of halothane and the binding of its reactive intermediates to hepatocellular macromolecules. This would be consistent with the proposed mechanisms of many hepatotoxic agents.[22]

Lactulose is known to protect against galactosamine-induced hepatic injury by a number of mechanisms. It causes an alteration in enteric flora via a reduction in stool *p*H resulting from its metabolism to acidic byproducts by the resident bacteria.[12] In addition, it is believed to interact directly with LPS, since it can inhibit the limulus amebocyte lysate assay for LPS *in vitro* and prevent galactosamine-induced necrosis *in vivo* by iv administration.[21] The protective action against HH-induced liver injury by lactulose pretreatment would implicate LPS as a factor in the development of hepatic necrosis in the HH model. It also would indicate that necrosis resulting from HH is via a chemotoxic mechanism, since similar pretreatment reduced the hepatic damage resulting from the classic hepato-chemotoxin galactosamine.[12]

Since halothane has been shown to reduce the phagocytic ability of human leukocytes *in vitro*,[23] we investigated the possibility that halothane anesthesia might alter the functional ability of the macrophages of the reticuloendothelial system that normally remove and detoxify LPS.[7] However, HH exhibited no effect on the ability of the reticuloendothelial system to phagocytize carbon particles *in vivo* in the rat at 2 h after anesthesia.

Hepatic calcium levels were examined as an indicator of the degree of damage or dysfunction of the hepatocytes.[16] Because LPS is known to bind to hepatocytes[24] and to interact with and decrease the fluidity of membranes,[25] alter membrane bound enzyme systems,[26] and open ion channels,[27] it is possible that membrane permeability alterations leading to altered hepatic calcium levels may be a mechanism by which LPS potentiates liver injury in the HH model. Surprisingly, a subnecrotic dose of LPS tended to increase hepatic calcium at 24 h after treatment to a greater level than that occurring with the hepatotoxic HH model. Lipopolysaccharide potentiation of HH caused an additive increase in hepatic calcium levels, while plasma GPT levels were increased synergistically. It would seem that LPS or perhaps a mediator released by macrophages[7] causes an increase in membrane permeability to calcium with minimal leakage of the cytosolic enzyme, GPT. However, in animals treated with LPS after HH there is a rapid loss of cytosolic enzyme as evidenced by the sudden increase in plasma GPT after LPS administration. These dramatic changes may result from the combined insult of halothane-initiated membrane damage and the interaction of LPS or its mediator with the compromised plasma membrane. It seems reasonable to suggest that membrane fluidity alterations caused by LPS would change the permeability of halothane-damaged cells to a point sufficient to result in increased cell death as evidenced by the greater efflux of cytosolic enzymes and the increased necrosis observed in the LPS-potentiated HH model.

The method of evaluating hepatic histopathology described here, hepatic percent MP infiltration, differs from that previously reported by this laboratory.[6] It does not contradict preceding work as evidenced by a very similar pattern in the time course of HH lesion development (fig. 3), as reported prior.[6] However, this method was utilized in order to obtain a more quantitative evaluation of actual hepatic necrosis rather than arbitrarily assigning a "score" on overall histologic appearance. This new technique of histologic evaluation was performed by an unblinded observer and awaits complete confirmation in future studies.

Since humans are known to be one of the most susceptible of all creatures to the deleterious effects of LPS,[28] these findings may well have pertinence in the human situation of halothane-induced hepatic damage. Although our observations in the rat HH model showed no changes in the rate of clearance of carbon particles from the blood after halothane anesthesia, surgery in humans has been shown to cause an inhibition of 20–50% in the phagocytic ability of the reticuloendothelial system that normally detoxifies LPS.[29] This decrease can persist from 1 to 6 days. Also, ischemia of the lower

gut or the release of histamine or serotonin by peritoneal macrophages during surgery could cause an enhanced absorption of endogenous LPS.[30] Thus, patients who have developed minor hepatic damage from halothane anesthesia could be at risk if they had a compromised ability to detoxify LPS and/or by greater than normal amounts of endogenous LPS being presented to the liver. A greater level of necrosis as well as the ability of LPS to cause an increased infiltration of macrophages and activated lymphocytes into the liver[31] would lead to an enhanced phagocytosis of halothane-altered hepatic tissue macromolecules. Since macrophages are known to process antigens for subsequent antibody production by lymphocytes,[32] this condition could well contribute to the fulminant, antibody-linked halothane hepatitis in individuals prone to an autoimmune reaction.

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