Title: ROLE OF ENDOTOXIN IN THE HALOTHANE HEPATITIS MODEL IN RATS

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Introduction. Endotoxins are lipopolysaccharides (LPS) derived from bacteria that reside in the lower G.I. tract. They are absorbed through the intestinal wall and presented to the liver, which has evolved as the primary organ for elimination of these potentially systemic toxins. LPS have been implicated as the causative factor in hepatic necrosis in rats caused by galactosamine[1] and $CCl_4$[2]. For this reason we investigated the role of endotoxins in halothane associated liver injury. Both the potentiation of necrosis by exogenous LPS and the prevention of hepatic damage with an antiendotoxin agent, lactulose, were examined in the rat hypoxic model.

Methods. Male Sprague-Dawley rats (300-350 g) were pretreated with phenobarbital to induce their liver microsomal enzymes. Certain of these received lactulose in their water (10% w/v) for 7 days (avg. consumption = 6.3 g/kg/day). The hepatic cytochrome P-450 content was determined for both the phenobarbital and the lactulose plus phenobarbital treatment regimes. Following these pretreatments, rats were exposed to 1% halothane for 2 hr at an $F_IO_2$ of 0.14. Controls were exposed only to $F_IO_2$ of 0.14. Animals receiving LPS were injected immediately after exposure with 0.5 mg/kg 026:B6 E. coli endotoxin via the tail vein. Twenty four hr post exposure the plasma GPT were determined. Liver sections were fixed, stained with H&E, and evaluated for: increased eosin staining, vacuolization, polymorphioneutrophil (PMN) infiltration, and coagulative necrosis by observing 6 random fields on each slide through a 100 square grid at 40X. Each parameter was quantified as the percent incidence in the fields observed.

Results. The hepatic cytochrome P-450 levels (1 nmole/mg microsomal protein) were similar in both the phenobarbital only and the lactulose plus phenobarbital treated animals. Rats receiving hypoxia + LPS displayed a slight increase in GPT over controls (Table) with the presence of only an occasional small necrotic area. GPT levels in rats treated with LPS after halothane-hypoxia (HH) exhibited a 4-fold increase over animals receiving HH only. Rats pretreated with lactulose prior to HH showed a 3-fold decrease as compared to the HH only group. The incidences of increased eosin staining of hepatocytes and vacuolization were similar between the HH and HH + LPS groups. Although HH + LPS animals showed a several fold increase in the occurrence of coagulative necrosis over the HH only rats, PMN infiltration proved to be a better means of comparison. The HH + LPS treatment group showed a 2-fold rise in PMN infiltration over HH alone while in the lactulose + HH group a 2-fold decrease was observed (Table).

Discussion. These results are in agreement with other reports in the literature in that chemical damage to the liver increases the susceptibility of liver to the necrogenic effects of LPS[1,3]. Exposure to halothane under hypoxic conditions lead to hepatocellular injury caused by reactive metabolites of halothane. Following this initial insult LPS may trigger actual cell death by a direct action or by complement activation and binding to the injured cells[1] (i.e. an enhanced inflammatory response). Our data supports this hypothesis, since equivalent degrees of increased eosin staining and vacuolization were found in the HH and HH + LPS groups, while LPS potentiated the inflammatory reaction (PMN infiltration) and necrosis. Therefore, any condition that increases the amount of LPS presented to the liver may potentiate halothane associated liver injury. Conversely, a reduction in hepatic endotoxin levels may prevent necrosis. Lactulose, a nonabsorbed sugar and established antiendotoxin agent,[4,5] afforded protection against halothane induced liver injury. Further studies are needed to demonstrate that circulating levels of LPS are enhanced under conditions that promote halothane associated liver injury. Such findings may help delineate the sequence of events that occur in this animal model of halothane associated liver injury.

TABLE: EFFECT OF ENDOTOXIN (LPS) AND LACTULOSE ON HALOTHANE HEPATOTOXICITY

| Treatment (N value)[a] | | GPT[b] | Necrosis | PMN[c] |
|---|---|---|---|---|
| Hypoxia | (6) | 22±4 | 0 | 0.5 |
| Hypoxia + LPS | (10) | 30±9 | + | 2.9 |
| HH | (21) | 100±66 | + | 9.8 |
| HH + LPS | (9) | 419±300 | +++ | 19.0 |
| Lactulose + HH | (8) | 31±17 | 0 | 4.4 |

[a] Hypoxia = 0.14 $F_IO_2$; HH = 1% halothane; 0.14 $F_IO_2$ 2 hr; phenobarbital induction

[b] mean ± SD

[c] mean % of grid field with PMN infiltration

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