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D-Galactosamine Liver Injury: Absorption of Endotoxin and Protective Effect of Small Bowel and Colon Resection in Rabbits (41555)

DANIEL S. CAMARA,[1] JOSEPH A. CARUANA, JR., KATHRYN A. SCHWARTZ, MARIO MONTES, AND JAMES P. NOLAN

Division of Gastroenterology, and Departments of Medicine, Surgery, and Pathology, Buffalo Veterans Administration Medical Center, Buffalo, New York 14215; and the State University of New York at Buffalo

Abstract. D-Galactosamine is an amino sugar with unique hepatotoxic properties in animals. Although the mechanism of liver injury by galactosamine remains controversial, a role for bacterial endotoxin has been suggested. In the present study, using New Zealand rabbits, we show that the significant increase in serum glutamic oxaloacetic transaminase which followed the injection of 4.25 mmole/kg of D-galactosamine was completely prevented in animals subjected to resection of small bowel and colon. Using an immunoradiometric assay specific for *E. coli* 026 endotoxin we showed that after instillation of 50 mg of *E. coli* into the colon, serum levels of this endotoxin were higher in the animals injected with galactosamine than the controls injected with saline. However, the differences in endotoxin concentration between the two groups of animals was statistically significant only at 30 and 60 min. The role of endotoxin in the pathogenesis of galactosamine liver injury is reviewed and discussed.

Since the discovery by Decker and Keppler of the hepatotoxic effects of D-galactosamine hydrochloride in rats (1, 2), this substance has provided a model for the study of fulminant liver necrosis in experimental animals. Galactosamine interferes with protein synthesis in the hepatocytes, but whether this is the mechanism by which the characteristic hepatocellular necrosis occurs has not been clarified. Endogenous gut-derived endotoxin has been suggested (3, 4) as the common mediator of hepatocellular injury induced by a variety of hepatotoxins. In both acute carbon tetrachloride (CCL_4) liver injury (5, 6) and cirrhosis of chronic choline deficiency (7), endogenous lipopolysaccharide (LPS) was shown to be critical for the development of the liver lesion. More recently endotoxin has also been implicated in the pathogenesis of the liver injury induced by galactosamine; colectomy in rats has been reported to protect against liver injury induced by this agent (8, 9). Other studies, however, have challenged the role of gut-derived endotoxin in the production of hepatic injury (10, 11). In this communication we report a similar experimental model to test the role of the intact gut in the development of galactosamine hepatitis, and to detect and quantify the absorption of endotoxin into the systemic circulation after galactosamine administration.

Materials and Methods. *Animals.* New Zealand male rabbits, weighing approximately 3 to 4 lb were used in all of the experiments.

Galactosamine. D-Galactosamine hydrochloride (Sigma Chemical Co., St. Louis, Mo.), was dissolved in phosphate-buffered saline (PBS) with pH adjusted to 6.8 with NaOH. In all experiments the galactosamine was injected into an ear vein at a dose of 4.25 mmole/kg. Control rabbits received a similar volume of PBS.

Endotoxin. Escherichia coli 026 LPS (lyophylized) was obtained from Difco (Detroit, Mich.) and dissolved in isotonic saline.

Immunoradiometric assay for E. coli 026 endotoxin. The assay has been described in detail elsewhere (12). In brief, IgG fraction of rabbit antibody to *E. coli* 026 endotoxin was labeled with ^{125}I. Polyvinyl chloride microtiter V-shaped plates were coated with 50 μl of unlabeled antibody (100 ng/μl) in 0.1 *M* carbonate buffer (pH 9.6) and incubated overnight at 4°. The wells were then washed three times (PBS–Tween 20) and 50 μl of radiolabeled antibody diluted with 1% bovine serum

[1] To whom all correspondence should be addressed.

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albumin (100,000 cpm/50 μl) was added, and the plates were incubated overnight at 4°. Each well was washed five times (PBS–Tween) and counted for 1 min in a γ scintillation spectrometer. A standard curve was plotted on double logarithmic graph paper. This curve plots known concentrations of 026 LPS against the ratio of $E_B/1 - E_B$. (E_B is the endotoxin bound and is derived from the test counts per minute (cpm) minus the blank cpm, divided by the total cpm added). This assay can detect 1 ng/ml *E. coli* 026 endotoxin in serum and shows no cross-reactivity with LPS from other Gram negative organisms.

Enzyme determination. Serum glutamic oxaloacetic transaminase (SGOT) was measured by a modification of the International Federation of Clinical Chemistry method using the DuPont ACA Clinical Analyzer.

Statistical analysis. Enzyme values and *E. coli* 026 endotoxin were plotted against time and were analyzed by Student's *t* test with a confidence level of 95% considered significant ($p < 0.05$).

Experimental design. (1) Evisceration studies. After an overnight fast, rabbits were sequentially numbered and anesthesized in pairs with intravenous sodium pentobarbital. The carotid artery was exposed, and an indwelling cannula inserted for blood pressure monitoring and blood sampling. Four milliliters of blood were withdrawn for determination of baseline SGOT values. The animals assigned an even number had a resection of the small bowel and colon from the third portion of the duodenum to the peritoneal reflection of the sigmoid colon, through a midline incision of the abdomen (evisceration). Due to a common blood supply, the colon could not be resected without producing ischemia in the remaining small bowel. The hepatic artery and portal vein drainage from the spleen, stomach, and pancreas were left intact. The odd-numbered animals had manipulation of the gut without evisceration. The animals were then placed in restraining cages until recovery from the anesthesia. Ten eviscerated and eleven sham-operated animals were injected via an ear vein with 4.25 mmole/kg of D-galactosamine hydrochloride. After galactosamine administration, 4 ml of blood was withdrawn from the carotid artery every 30 min for 3.5 hr or until the animal expired. The mean blood pressure was continuously monitored, and serum samples were analyzed for SGOT.

After termination of the experiment, animals were sacrificed with an overdose of pentobarbital, and wedge liver biopsies were obtained. The liver biopsies were fixed in 10% buffered formaldehyde and stained with hematoxylin and eosin for histological examination.

(2) Absorption studies. Fasted rabbits were anesthesized in pairs and prepared as just described. After obtaining baseline values for SGOT and *E. coli* 026 LPS, a midline abdominal incision was performed and the colon exposed. Fifty milligrams of *E. coli* 026 LPS dissolved in 5 ml of isotonic saline was then injected into the lumen of the colon. Care was taken to avoid extravasation of the solution. The abdomen was closed, and the animals were allowed to recover from anesthesia. The 10 even-numbered animals received 4.25 mmole/kg of D-galactosamine via an ear lobe vein (Group 1). The 10 odd-numbered animals received the same volume of saline intravenously (Group 2). Blood was sampled every 30 min for 210 min for determination of *E. coli* 026 LPS concentration and SGOT activity. At the end of this period the animals were sacrificed with an overdose of sodium pentobarbital, and a wedge liver biopsy was obtained for histologic examination.

Results. *(1) Evisceration studies.* In the sham-operated rabbits given galactosamine (Group 1), there was an elevation of SGOT activity in the arterial blood which began at 90 min and continued throughout the period of observation (210 min). Animals subjected to evisceration showed no elevation of SGOT activity. The difference in SGOT activity between sham-operated and eviscerated animals was statistically significant ($p < 0.05$) at each time period beyond 120 min (Fig. 1).

The mean blood pressure was not different between sham-operated animals and eviscerated animals during the period of observation. Histological examination of the liver biopsies of these animals showed minimal focal necrosis without a consistent pattern in animals of both groups.

(2) Absorption studies. E. coli 026 endotoxin was readily absorbed from the colon into

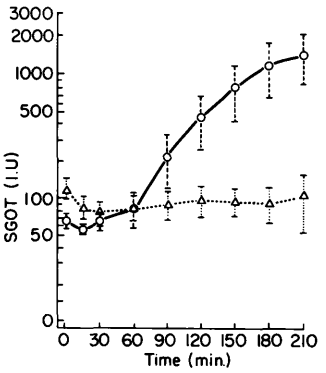

FIG. 1. Serum glutamic oxaloacetic transaminase (SGOT) activity (mean ± SEM) after administration of 4.25 mmole/kg of galactosamine into sham-operated animals (O) and animals with colon and small bowel resection (Δ). Differences between means are statistically significant, $p < 0.05$, at 120 min and thereafter.

the blood of animals given either D-galactosamine or isotonic saline. The difference in concentration of LPS between the two groups of animals was statistically significant only at 30 and 60 min ($p < 0.05$). After 90 min, the concentration of serum endotoxin in both groups was similar (Fig. 2). The SGOT activities were significantly higher than controls ($p < 0.05$) beyond 90 min in the group of rabbits receiving galactosamine (Fig. 2). There was no statistically significant difference in the blood pressures between the two groups of animals.

Discussion. In this study we demonstrate that colectomy and small bowel resection protects the liver against the early toxic effects of galactosamine, which otherwise produces a steady rise in SGOT activity in intact animals within the first 210 min of administration. These studies confirm the work of Liehr and associates who implicated endotoxin in the pathogenesis of this lesion (8, 9, 15, 16). Using the Limulus gelation test, they found that endotoxemia regularly follows the administration of galactosamine in normal rats and that the "endotoxin tolerant" state protected these animals from liver damage (9). In further studies by this group rats subjected to colectomy (9) or partial hepatectomy (17) were similarly protected. Complement-deficient rats (NMRI strain) were also refractory to the toxic effects of galactosamine (8). Based on these findings, they postulated that galactosamine renders the liver incapable of effectively detoxifying endotoxin. In addition, galactosamine was found to cause mast cell degranulation in the gut with local release of histamine, leading to an increase in the colonic absorption of endotoxin. The increased absorption of gut-derived endotoxin will then exert toxic effects on liver cells either directly or through the release of mediators by the Kupffer cells (3, 4, 18–20).

These conclusions, however, have been contested by recent studies. In isolated perfused rat livers where the colon is not a factor, galactosamine exerted a direct toxic effect on hepatocytes (10). Al-Tuwaijri *et al.*, using the Limulus gelation test, failed to confirm the presence of endotoxin in plasma after galactosamine administration in rats (11).

Decker and Keppler (1, 2) extensively studied galactosamine liver injury and have observed an intracellular depletion of uridine triphosphate (UPT) shortly after galactosamine administration with a resulting accumulation of uridine diphosphate (UDP). They speculated that the decrease in UTP levels interfered with RNA synthesis with subsequent inhibition of the synthesis of proteins and

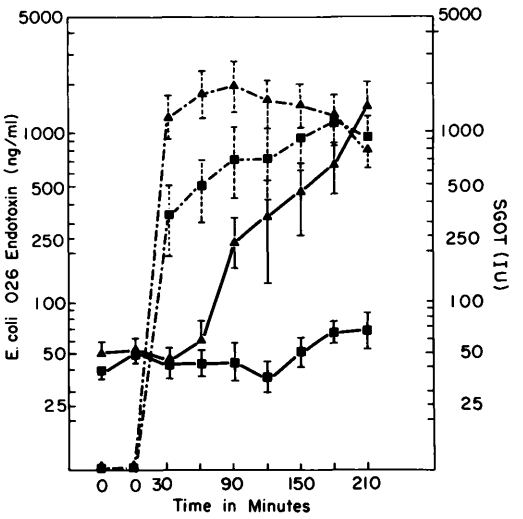

FIG. 2. SGOT activities (——) and serum *E. coli* 026 endotoxin concentration (-·--·) in rabbits infused with galactosamine (▲) or saline (■). *E. coli* 026 endotoxin levels were significantly higher ($p < 0.05$) in the rabbits receiving galactosamine at 30 and 60 min. SGOT activity was significantly higher in the galactosamine group beyond 90 min.

other macromolecules (1). El-Mofty *et al.* (13) postulated that the changes in level of intracellular UDP-hexosamines were responsible for substantial structural changes in the plasma membrane of the hepatocytes leading to cell necrosis. However, other drugs that induce similar inhibition of protein synthesis, e.g., ethionine (13), cycloheximide (14), and actinonycin D (9), do not cause significant hepatocellular necrosis. This fact as well as the severity of the liver injury suggested alternate explanations for the hepatotoxic effects of galactosamine.

The SGOT activity has been shown to reflect the magnitude of the liver cell injury and/or necrosis after galactosamine administration. Blitzer *et al.* as well as Decker and Keppler (1, 2) have carefully quantitated the liver injury induced by galactosamine using a battery of serum markers such as: SGOT, glutamic pyruvic transaminase, lactate dehydrogenase, sorbitol dehydrogenase, fibrinogen, prothrombin, ammonia, and amino acids (21). They found no histological evidence of injury in other areas rich in SGOT such as the heart and striated muscle. Therefore, in the present study we considered that the SGOT values accurately reflected early hepatocyte injury.

The histological changes observed in the liver after galactosamine administration have been extensively studied (1, 2, 14, 26, 27). The lack of significant histological differences between controls and animals which received galactosamine in our experiments is not surprising since the time frame used in this study was too brief for significant morphological changes to occur. Indeed, Koff *et al.* observed by light microscopy the first signs of histologic damage post-galactosamine administration only after 4 hr (27).

It should be mentioned that evisceration can produce hemodynamic changes not detected by measuring the blood pressure alone. However, in preliminary studies we did not observe significant elevation of SGOT activity 24 hr after resecting the small bowel and colon in rabbits. Furthermore, after evisceration, not only is there a marked reduction in the amount of endogenous endotoxin, but also in the level of splanchnic hormones and metabolic products such as ammonia and bile acids. The role of these factors has not been evaluated in the pathogenesis of galactosamine liver injury.

In the absorption studies we used an immunoradiometric assay for *E. coli* 026 endotoxin rather than the Limulus assay (22–24). This assay is specific for *E. coli* 026 LPS which is not present in the rabbit colon, thus serving as a marker for endotoxin absorption. We observed that endotoxin was readily absorbed from the colon and detected in the blood of the animals injected with galactosamine and the controls injected with saline. The absorption of endotoxin could be due in part to a nonspecific depression of the reticuloendothelial system secondary to anesthesia (25). However, despite the similar serum concentration of LPS in both groups of animals, SGOT elevation was observed only in the animals injected with galactosamine. We believe that galactosamine may render the liver susceptible to the toxic effects of endotoxin. This conclusion is further supported by the first part of our experiment where removal of the colon (resevoir of endotoxin) neutralized the toxicity of galactosamine to the liver. Whether the endotoxemia detected in these animals represents increased absorption, decreased detoxification of LPS by the liver, or a combination of both could not be determined in this study.

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