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SHORT COMMUNICATION

Minimal Effect of Acute Experimental Hepatitis Induced by Lipopolysaccharide/ D-Galactosamine on Biotransformation in Rats

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ABSTRACT. When administered with D-galactosamine, lipopolysaccharide endotoxins produce a good experimental animal model of hepatitis. This galactosamine plus endotoxin model has been used widely, but the acute effect of this fixed combination of two chemicals on hepatic and extrahepatic biotransformation has not been determined. Therefore, either 2 or 4 hr after a single intraperitoneal dose of 300 mg/kg galactosamine plus 30 μg/kg lipopolysaccharide was administered, serum, liver, kidney, intestine, and spleen were collected. Serum enzymes (alanine and aspartate aminotransferases, sorbitol dehydrogenase, and γ-glutamyltranspeptidase) were elevated dramatically 2 and 4 hr after treatment. Cytochrome P450 monooxygenase activity toward benzo-[a]pyrene was increased in kidney 4 hr after treatment, whereas dealkylation of 7-methoxycoumarin or 7-ethoxyresorufin was unchanged in any tissue at either time point. An increase in UDP-glucuronosyltransferase activity toward 4-methylumbelliferone and 4-hydroxybiphenyl was noted in the intestine. Conjugation of 1-chloro-2,4-dinitrobenzene with glutathione was increased in intestine and spleen 2 hr after treatment. γ-Glutamyltranspeptidase activity was unaltered in all tissues studied. Reduced glutathione concentrations were increased significantly by different amounts depending on which organs were studied 2 or 4 hr after treatment. These results indicate that galactosamine/lipopolysaccharide-induced liver injury is not accompanied by major effects on the examined biotransformation reactions. *Copyright © 1996 Elsevier Science Inc.* BIOCHEM PHARMACOL 52;12:1921–1924, 1996.

KEY WORDS. UDP-glucuronosyltransferase; cytochrome P450 monooxygenases; liver; kidney; spleen; intestine; endotoxin; galactosamine

Small amounts of endogenous endotoxin are absorbed regularly and detoxified rapidly by the liver [1]. Impairment of LPS† degradation may accentuate existing hepatic damage by allowing toxic levels of endotoxin to accumulate in hepatic tissue, and by allowing LPS entry into the systemic circulation, leading to extrahepatic effects [2].

A well-established model of hepatitis takes advantage of the ability of GalN to potentiate the toxic effects of LPS, producing fulminant hepatitis within a few hours of administration [3]. This dose regimen with GalN and LPS typically causes lethality within 5–9 hr [3]. While there are conflicting data as to whether LPS alone alters hepatic activity of cytochrome P450-dependent monooxygenases and UDP-glucuronosyltransferases [4–6], no one has examined hepatic or extrahepatic biotransformation in this model. Therefore, the present work has determined the activities of specific cytochrome P450-dependent monooxygenases, UDP-glucuronosyltransferases, glutathione S-transferases and GGT from liver, kidney, intestine, and spleen in rats treated with 300 mg/kg GalN plus 30 μg/kg LPS.

MATERIALS AND METHODS

Chemicals

LPS from *Salmonella abortus equi* and all other chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO). Deionized water was used in all studies.

Animal Treatment, Tissue Preparation, and Enzyme Assays

Male Sprague–Dawley rats (275–325 g, Harlan Sprague Dawley, Inc., Indianapolis, IN) were provided Purina rat chow (No. 5012) and water *ad lib.* until used. Rats received a single intrapertioneal administration of 300 mg/kg D-GalN plus 30 μg/kg LPS in a total volume of 1 mL phosphate-buffered saline (pH 7.4) per rat. Control animals were injected with 1 mL buffer only. Because of the significant mortality associated with this paradigm, half of the treated animals were euthanized with pentobarbital (40 mg/

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† *Abbreviations:* ALT, alanine aminotransferase; AST, aspartate aminotransferase; CDNB, 1-chloro-2,4-dinitrobenzene; GalN, galactosamine; GGT, γ-glutamyltranspeptidase; GSH, reduced glutathione; LPS, lipopolysaccharide; and SDH, sorbitol dehydrogenase.

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kg) after 2 hr and the rest along with the control rats after 4 hr. Blood was collected via cardiac puncture, and serum activities of AST, ALT, SDH, and GGT were determined using diagnostic kits from the Sigma Chemical Co. Liver, kidneys, intestine, and spleen were removed and washed in ice-cold 1.15% KCl solution. Kidneys were decapsulated, the intestines were opened and cleaned, and then all tissues were frozen for subsequent analysis. Using a Brinkmann Polytron homogenizer, 1% homogenates (w/v) in 0.1 M Tris–HCl buffer (pH 7.4) were prepared for assaying cytochrome P450 monooxygenase, UDP-glucuronosyltransferase, and GGT activities. Cytochrome P450 monooxygenase activity was determined fluorometrically: deethylation of 50 μM 7-ethoxyresorufin was determined with 200–400 μg protein in a 30-min incubation [7]; hydroxylation of 100 μM benzo[a]pyrene was measured with 600–900 μg protein after a 15-min incubation [8]; and demethylation of 400 μM 7-methoxycoumarin was quantitated after a 5-min incubation with 200–400 μg protein [9]. UDP-glucuronosyltransferase activities were assayed fluorometrically with 600–900 μg protein and either 500 μM 4-hydroxybiphenyl [10] or 500 μM methylumbelliferone [11] as substrates. GGT activity was determined by the method of Meister *et al.* [12]. Cytosols (5%, w/v; 100,000 *g* supernatant) with 50–75 μg protein were used for spectrophotometric determination of glutathione *S*-transferase conjugation of 2 mM CDNB [13]. A 2% (w/v) homogenate in 0.1 M formic acid was used for fluorometric determination of GSH [14]. Total protein concentrations in homogenates and cytosols were determined by the method of Lowry *et al.* [15].

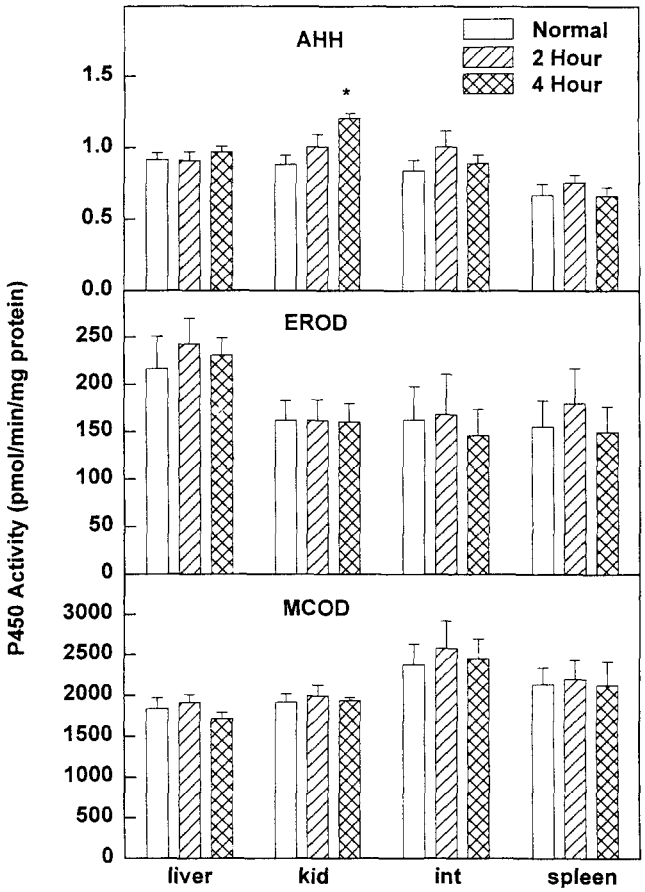

FIG. 1. Acute effect of GalN/LPS on cytochrome P450 monooxygenase activity, hydroxylation of benzo[a]pyrene (AHH), deethylation of 7-ethoxyresorufin (EROD), and demethylation of 7-methoxycoumarin (MCOD) in liver, kidney (kid), intestine (int), and spleen 2 and 4 hr after treatment. Bars represent means ± SEM of 8 rats. Key: (*) significantly different ($P < 0.05$) from normal.

RESULTS AND DISCUSSION

Although numerous studies of hepatitis have used the GalN/LPS model [16–18], no systematic evaluation of the combined effect of the acute action of these two chemicals on biotransformation has been made in liver or other organs. Hepatic injury induced by GalN/LPS was demonstrated by markedly elevated serum activities of AST (normal, 95 ± 11; 2-hr, 323 ± 21; 4-hr, 529 ± 61 Sigma U/mL serum), ALT (normal, 100 ± 10; 2-hr, 275 ± 25; 4-hr, 657 ± 111 Sigma U/mL serum), SDH (normal, 202 ± 12; 2-hr, 1210 ± 69; 4-hr, 3000 ± 615 Sigma U/mL serum), and GGT (normal, 1010 ± 210; 2-hr, 1870 ± 270; 4-hr, 2880 ± 243 μmol/mL serum). Examination of hematoxylin and eosin-stained liver samples demonstrated scattered necrosis and infiltration of inflammatory cells.

Endotoxin alone has been found to stimulate the degradation of cytochrome P450 in rat liver within hours [19]. In humans, LPS decreases hepatic cytochrome P450-mediated drug metabolism over a 24-hr period [6]. In contrast, GalN plus LPS did not reduce the microsomal hydroxylation of benzo[a]pyrene, deethylation of ethoxyresorufin, or demethylation of 7-methoxycoumarin by liver, kidney, intestine, and spleen (Fig. 1). The mechanism for the 37% increase in benzo[a]pyrene hydroxylase activity that was noted only in kidney after 4 hr is unknown.

Intestinal UDP-glucuronosyltransferase activity toward 4-methylumbelliferone and 4-hydroxybiphenyl was increased 5- and 2-fold, respectively, 4 hr after GalN/LPS (Fig. 2). There were no other statistically significant changes in the glucuronidation of these substrates in the other tissues. In contrast to these minimal alterations in the glucuronidation of 4-methylumbelliferone and 4-hydroxybiphenyl in GalN/LPS-induced hepatitis, numerous substrate-dependent alterations in UDP-glucuronosyltransferase activity occur after the spontaneous development of hepatitis about 16 weeks after birth in the Long Evans Cinnamon-like coat color rat [20]. Increasing intestinal glucuronosyltransferase activity may help in the conjugation of LPS or its altered products which leave the hepatocyte via the bile duct and enter the gut [21].

Glutathione *S*-transferase activity toward CDNB increased 2-fold in intestine and spleen 2 hr after treatment with GalN plus LPS (Fig. 3, top). There were no significant changes at 4 hr in any tissue. Concentrations of GSH were

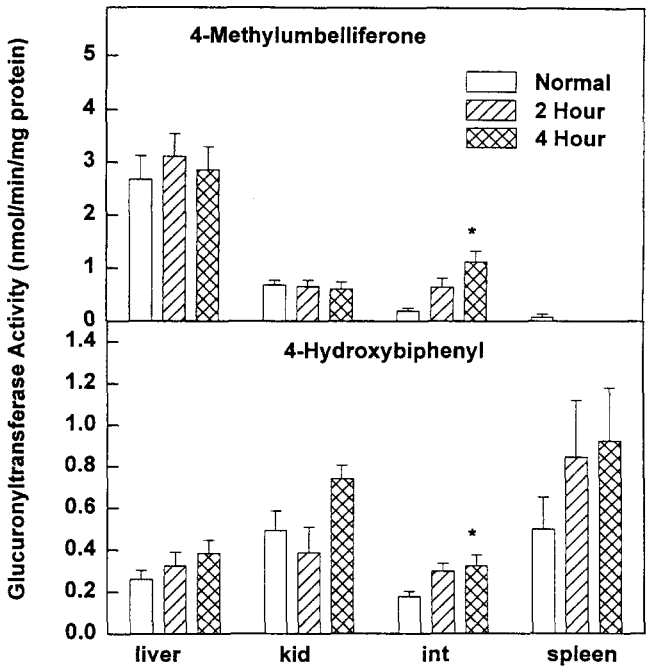

FIG. 2. Acute effect of GalN/LPS on UDP-glucuronosyltransferase activity toward 4-methylumbelliferone and 4-hydroxybiphenyl in rat liver, kidney (kid), intestine (int) and spleen, 2 and 4 hr after treatment. Bars represent means ± SEM of 8 rats. Key: (*) significantly different ($P < 0.05$) from normal.

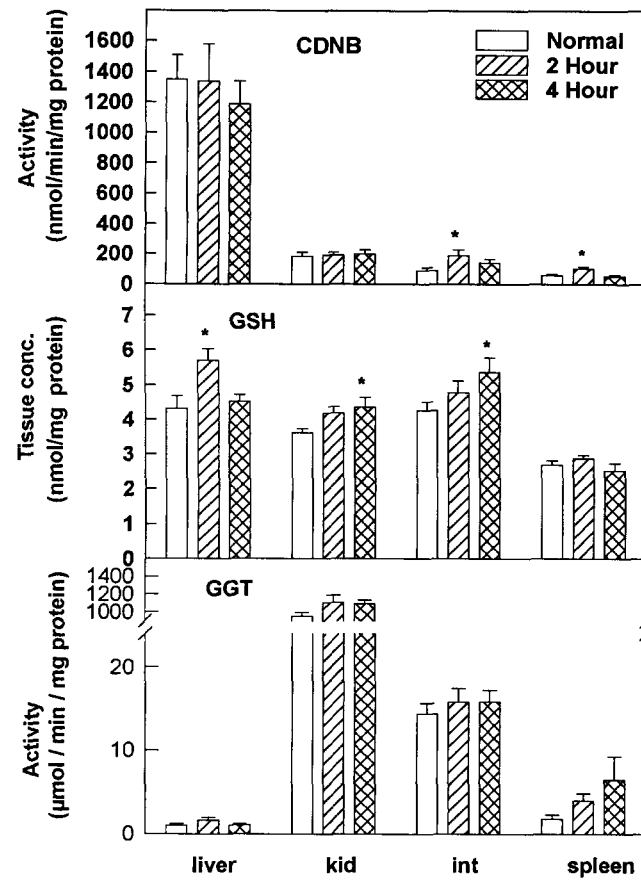

FIG. 3. Acute effect of GalN/LPS on glutathione S-transferase activity toward CDNB (top), reduced glutathione (GSH) concentrations (middle), and γ-glutamyltransferase (GGT) activity (bottom) in rat liver, kidney (kid), intestine (int), and spleen. Bars represents means ± SEM of 8 rats. Key: (*) significantly different ($P < 0.05$) from normal.

increased significantly in liver after 2 hr, and in kidney and intestine 4 hr after treatment with GalN and LPS (Fig. 3, middle). There was no change in GSH in spleen. Certain carcinogens increase GSH levels and the activities of GGT and glutathione S-transferases, and electrophilic reactive forms of carcinogens may be conjugated with GSH [22, 23]. Moreover, LPSs have the ability to induce the release of acid-soluble thiols from macrophages, which could constitute a protective mechanism against oxidative stress and tissue injury [24]. GGT activity (Fig. 3, bottom) was not significantly different in any treatment group in any organs tested.

The liver and kidney are largely responsible for inactivation and elimination of drugs and other chemicals. UDP-GalN derivatives, which begin accumulating in liver within 30 min of GalN administration [16], return to the blood stream and are filtered by the kidney. However, the kidney does not detoxify endotoxin [25]. Intestinal conjugation represents an early defense of the organism against orally ingested xenobiotics, in some cases exceeding conjugation in the liver [26].

There have been only a few reports regarding biotransformation reactions in spleen [27, 28]. Apparently, spleen has levels of cytochrome P450 monooxygenases similar to that in liver (Fig. 1). Our results in Fig. 2 show a low level of activity in normal and GalN/LPS-treated rat spleen toward 4-methylumbelliferone, and a markedly higher activity toward 4-hydroxybiphenyl. In addition, conjugation of CDNB with glutathione was very low in spleen (about 5% of that in liver, Fig. 3), but was elevated in intestine and spleen 2 hr after treatment with GalN/LPS. This may be due to the high susceptibility of the gastrointestinal cells to the carcinogenic actions of various xenobiotics [29]. Finally, GGT activity in spleen was similar to that in liver in normal and GalN/LPS-treated rats (Fig. 3).

In conclusion, GalN/LPS-induced liver injury is not accompanied by significant alterations in biotransformation by liver, kidney, intestine, or spleen.

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