MICROSOMAL DRUG METABOLISM IN ACUTE AND CHRONIC GALACTOSAMINE INDUCED HEPATIC INJURIES

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Summary

To determine the microsomal functions of the injured liver, female rats were given D-galactosamine (GaN) acutely (400 mg/kg body weight, i.p.) or chronically (250 mg/kg body weight for 7 months, daily, i.p.). In the acute study, GaN administration resulted in a five-fold increase in hepatic triglyceride content. Microsomal protein and phospholipid concentrations were not affected. However microsomal cytochrome P450 content and aminopyrine demethylase and also aniline hydroxylase activities were significantly decreased whether expressed per mg of microsomal protein or per gram of liver. Cytochrome b5 content was significantly increased. By contrast, chronic administration of GaN produced a marked hepatomegaly and an extensive hepatic fibrosis with nodular formation without triglyceride accumulation. Electron-microscopically, there was apparent proliferation of mitochondria, and smooth endoplasmic reticulum showed focally accentuated hypertrophy without liposomes in the dilated tubules. Although microsomal protein concentration was significantly decreased, cytochrome P450 content was increased significantly when expressed per mg of microsomal protein or per unit of body weight. The aminopyrine demethylase activity was significantly enhanced only when expressed per unit of body weight. In both acute and chronic studies, microsomal glucose-6-phosphatase activity was significantly reduced. These data suggest that in acute liver injury, there is an impairment of hepatic microsomal drug metabolizing activity, whereas in chronic liver injury, there is an apparent enhancement of the activity possibly due to an enlarged hepatic mass even in the presence of a decreased drug metabolizing capacity per unit of the injured liver.

Key Words: galactosamine, microsomal drug metabolism, liver cirrhosis.

D-galactosamine (GaN), when given acutely to rats, is known to produce extensive hepatocellular necrosis histologically similar to that of human viral hepatitis1 with concomitant fatty degeneration2,3. Furthermore electronmicroscopic studies disclosed an extensive hypertrophy of the smooth endoplasmic reticulum (SER) and other alterations in the liver when GaN was administered acutely2-4. Although drug induced hypertrophy of the endoplasmic reticulum is generally associated with increased activity of the microsomal drug metabolizing system, GaN-induced hypertrophy of SER has been shown not to be associated with an increased drug metabolizing activity4-8. Subsequently Lesch et al.9 reported that long term intraperitoneal administration of GaN to rat resulted in hepatic fibrosis or hepatic cirrhosis in about 6 months. However, there have been no reports on microsomal functions of the liver chronically treated with GaN.
Since drug metabolizing activity in chronic liver disease is still a matter of debate, we attempted to determine whether both acute and chronic administration of GalN affect the biochemical components and drug metabolizing enzyme activities of the microsomal membrane. Histological study was also carried out on chronic GalN-induced liver injury in an attempt to correlate with biochemical findings.10

Material and Methods

D-galactosamine hydrochloride (95% purity) was generously supplied by Research Laboratories of Chugai Pharmaceutical Co. Ltd., Japan.

Wistar strain female rats weighing about 200 grams were used for the experiments. They were fed standard laboratory chow diets (Oriental Yeast Mfg. Ltd., Japan) and drinking water ad libitum.

In the acute study, seven rats were given single intraperitoneal injection of neutralized 20% D-Galactosamine hydrochloride solution with a dose of 400 mg/Kg body weight 24 hours before sacrifice. In the chronic study, seven rats were given daily intraperitoneal injections of neutralized 20% D-Galactosamine hydrochloride solution with a dose of 250 mg/Kg body weight for 7 months. Control rats were given equal volume of physiological saline. All animals were fasted 12 hours before sacrifice by decapitation. A small portion of the liver was immediately extirpated, weighed and prepared for the light and electron microscopy. The rest of the livers were perfused with ice-cold physiological saline and quickly extirpated, weighed and used for the biochemical experiments.

All subsequent steps were performed at 0—4°C. Livers were homogenized with three volumes of 0.25 M sucrose solution using a glass homogenizer with teflon pestle. The homogenates were centrifuged at 10,000 g for 20 minutes in a high speed refrigerated centrifuge (Tomii Co. Ltd., Japan). The total microsomal fractions were obtained by centrifugation of the 10,000 g supernates with Beckman L5—50 ultracentrifuge using Type 40 rotor at 100,000 g for 60 minutes. The pellets were resuspended in 0.25 M sucrose solution and used for chemical analyses and enzyme assays.

Microsomal protein was determined according to Lowry et al.11

Hepatic triglyceride concentration was determined according to Morita12. Microsomal lipids were extracted according to Folch et al.13 and lipid phosphorus was determined by the method of Bartlett14. The amount of phospholipids was calculated by multiplying the amount of lipid phosphorus by 25. Cytochrome P450 content was measured according to Omura and Sato15, and cytochrome b5 content according to Strittmatter et al.16 using Shimadzu MPS-5000 double beam spectrophotometer. NADPH cytochrome C reductase activity was measured as described by Masters et al.17. Microsomal N-demethylase activity for aminopyrine was measured by the method of Holtzman et al.18 and formaldehyde produced was determined according to Nash19. Microsomal aniline hydroxylase activity was determined by measuring the quantity of p-aminophenol produced by the reaction20. Glucose-6-phosphatase activity was measured according to Harper21.

The preparative loss of microsomes was corrected by the method of Greim22. Corrections were made by comparing the contents of cytochrome P450 per gram of liver in whole homogenates and obtained microsomes.

Specimens for light microscopy were observed by hematoxylin eosin and other stains. For electron microscopy small pieces of liver