Microsomal Cytochrome P-450 Monoxygenase System and Its Drug-metabolizing Activity after Partial Portal Vein Ligation in the Rat

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Abstract. Percutaneous transhepatic portal vein embolization (PTPE) has been used to decrease the risk of hepatic failure after hepatectomy in patients with poor liver function. The effect of PTPE on hepatic drug-metabolizing activities is not clear. Therefore we examined the effect of portal vein branch ligation, a model of PTPE, on hepatic drug-metabolizing activities in Sprague-Dawley rats. Ligated and nonligated lobes were harvested separately. Drug-metabolizing activities and concentrations of components of the microsomal cytochrome P-450 monoxygenase system were examined. In ligated lobes, drug-metabolizing activities (lidocaine and aminopyrine) and enzymatic concentrations of the microsomal cytochrome P-450 monoxygenase system gradually decreased over 10 days. In nonligated lobes these functions were depressed rapidly to 60% of those before PBL but then recovered 10 days after PBL. From the viewpoint of drug metabolism, hepatic dysfunction occurred in both ligated and nonligated lobes.

Hepatic insufficiency is a serious complication of extended hepatectomy. The remnant liver’s regenerative capacity is an important factor in the survival of patients after hepatectomy. Recently, the portal vein branch supplying the area to be resected has been embolized preoperatively to reduce the risk of hepatic failure after hepatectomy [1, 2]. Patients receive various drugs during this preoperative period, but the effect of percutaneous transhepatic portal vein embolization (PTPE) on hepatic drug-metabolizing activity is not clear.

The microsomal cytochrome P-450 monoxygenase system of the liver is responsible for biotransformation of drugs and other lipophilic compounds into polar metabolites, a process essential for excretion in bile or urine [3]. During this process, molecular oxygen is incorporated into substrates by cytochrome P-450, a terminal electron donor that receives electrons from NADPH or NADH through cytochrome P-450 reductase or cytochrome b5 and cytochrome b2 reductase, respectively [4]. In this experiment we examined the effects of portal vein branch ligation (PBL), a model of PTPE, on liver microsomal drug-metabolizing activities and the cytochrome P-450 monoxygenase system.

Materials and Methods

Animals

Thirty-six male Sprague-Dawley (SD) rats weighing 200 to 300 g were used. All animals were housed in our animal facility and fed rat chow and tap water ad libitum.

Experimental Design and Surgical Procedures

Thirty rats were divided into five groups (0, 1, 3, 6, and 10 days after PBL) and used to study the effects of PBL. Rats were anesthetized with peritoneal injection of sodium pentobarbital 50 mg/kg.

For PBL, the portal vein branches (70% of total liver) of the left lateral and median lobes were separated from the hepatic artery and bile duct and ligated. The abdominal cavity was closed, and the rats were kept alive for up to 10 days. The ligated and nonligated lobes were harvested separately at 0, 1, 3, 6, and 10 days after PBL; and microsomes were prepared for analysis. To examine the possible effects of laparotomy, identical analyses were done in six sham-operated rats.

Preparation of Liver Microsomes

All procedures were performed at 4°C. The liver was homogenized, and the microsomes were prepared with the method of Mitoma et al. [5]. Microsomal fractions were stored at −80°C for further analysis.

Protein Concentration

Protein concentrations in the microsomal suspension were estimated with the method of Bradford with a protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA) [6].
Cytochrome P-450, Cytochrome b₅, NADH-Cytochrome b₅ Reductase, and NADPH-Cytochrome P-450 Reductase

The concentrations of cytochrome P-450 and cytochrome b₅ were measured spectrophotometrically with molar extinction coefficients of 91,000 and 185,000, respectively [7]. The concentrations of NADH-cytochrome b₅ reductase and NADPH-cytochrome P-450 reductase were estimated with their ferricyanide reductase activities. The NADH-ferricyanide reductase activity of NADPH-cytochrome b₅ reductase and the NADPH-ferricyanide reductase activity of NADPH-cytochrome P-450 reductase were measured by the decrease in absorbance of ferricyanide at 420 nm [8].

Drug-Metabolizing Activities

The activities of aminopyrine N-demethylase and lidocaine N-deethylase were assayed as follows.

Aminopyrine N-Demethylase Activity. The reaction mixture in 1.5 ml of 50 mM Tris-HCl buffer pH 7.4 containing 5 mM of MgCl₂, 5 mM of glucose-6-phosphate, 0.5 mM of NADP⁺, 1.5 units of glucose-6-phosphate dehydrogenase, and about 1 mg protein/ml of microsomal suspension was preincubated for 5 minutes at 37°C. The enzyme reaction was started by adding 100 µl of 30 mM aminopyrine for aminopyrine N-demethylase. The reaction mixture was incubated in a shaking waterbath at 37°C for 15 minutes. Respective enzyme activities were calculated with the concentrations of released formaldehyde [9].

Lidocaine N-Deethylase Activity. Lidocaine N-deethylase activity was assayed by the formation of monooethyleglycinexylidide (MEGX) (Fujisawa Pharmaceutical, Osaka, Japan) [10]. Microsomal protein, 125 to 250 µg, was preincubated in potassium phosphate buffer 0.1 mol/L pH 7.4 containing 5 mM of MgCl₂, 5 mM of glucose-6-phosphate, 0.5 mM of NADP⁺, and 1 unit of glucose-6-phosphate dehydrogenase in a final volume of 1 ml for 5 minutes at 37°C. The enzyme reaction was started by the addition of 7 mM lidocaine. The reaction mixture was incubated for 15 minutes at 37°C in a shaking waterbath. The reaction was stopped by adding 0.1 ml of 60% perchloric acid (HClO₄). Denatured protein was removed by centrifugation (5 minutes at 10,000 rpm), and a 30-µl aliquot of the supernatant was injected for high-performance liquid chromatographic (HPLC) analysis.

HPLC Analysis. The HPLC system consisted of a pump (model 510; Waters Co., Milford, MA, USA), an autosampler (model 712; Waters), and a variable-wavelength ultraviolet detector (model 484; Waters) that was operated at 205 nm. The column (4.6 × 250 mm) was packed with Nucleosil 5-C-18 (Wako Pure Chemical Industries, Osaka, Japan). The mobile phase consisted of 4.2 mM acetic acid, 1.7 mM butylamine, and 10% (v/v) acetonitrile; it was delivered at 1 ml/min. Chromatograms were integrated with a integrator (model 741; Waters).

Concentration of Cytochrome P-450 3A2

The content of cytochrome P-450 3A2 was estimated with Western blotting using a cytochrome P-450 3A2 commercial kit (Daichi Pure Chemical Co., Tokyo, Japan). Microsomal proteins before PBL and 3 days after PBL were separated with polyacrylamide gel electrophoresis and transferred onto nitrocellulose paper. The separated cytochrome P-450 3A2 was then stained with a rabbit anti-rat cytochrome P-450 3A2 antibody and sheep antirabbit immunoglobulin G (IgG) antibody conjugated with horseradish peroxidase (Wako). The absorption of the cytochrome P-450 3A2 band was measured densitometrically with a dual-wavelength chromatoscaner (model CS-910; Shimadzu Co., Kyoto, Japan) to estimate its content. Values relative to the mean value (cm²/mg protein) before ischemia are shown.

Statistical Analysis

In the groups of ligated and nonligated livers, significance was tested with one-way analysis of variance. Differences between the groups at specific time points were considered significant at p < 0.05 with Dunnett’s test as a post hoc analysis. Values of ligated and nonligated livers at each time after PBL were compared with Student’s t-test. Differences with p < 0.05 were considered significant. All measurements are presented as means ± standard deviation.

Results

Changes in Liver Weights for 10 Days after PBL

The PBL procedure led immediately to significant atrophy of ligated lobes and to significant hypertrophy of nonligated lobes (Fig. 1).

Reduction in Activity of Cytochrome P-450 Monoxygenase System after PBL

In ligated lobes the activities of lidocaine N-deethylase (Fig. 2A) and aminopyrine N-demethylase (Fig. 2B) gradually decreased from 2.38 ± 0.33 to 0.92 ± 0.21 nmol/mg protein/min and from 4.28 ± 0.53 to 2.32 ± 0.30 nmol/mg protein/min, respectively, over 10 days. In nonligated lobes the activities decreased significantly to 0.93 ± 0.19 and 2.34 ± 0.50 nmol/mg protein/min, respectively, at 3 days after PBL and then returned to baseline levels by 10 days. Enzyme activities in ligated lobes and nonligated lobes differed significantly 1, 6, and 10 days after PBL.