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Effect of obstructive jaundice on the fate of a nephrophilic organic anion in the rat

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Renal transtubular transport of phenolsulfophthalein (PSP), a nephrophilic organic anion that circulates bound to albumin, was studied in normal and bile-duct-ligated rats. Intravenously injected PSP disappeared from the circulation more rapidly in bile-duct-ligated jaundiced rats than in intact animals. However, urinary excretion of PSP was significantly lower in the former than in the latter. Kinetic analysis revealed that binding of PSP to plasma protein(s) was significantly lower with jaundiced rats than with intact animals. Addition of albumin to plasma samples from bile-duct-ligated rats markedly increased PSP binding. The decreased PSP binding retured to normal levels after treating the jaundiced plasma with bilirubin oxidase, an enzyme that degrades amphiphilic bilirubin to water soluble metabolites. These results suggest that bilirubin might be the major metabolite that occupied the PSP binding site(s) on albumin in jaundiced rats. When PSP was injected bound to equimolar amount of albumin, the rate of PSP disappearence from the circulation decreased and urinary excretion of the ligand increased markedly; urinary excretion of PSP was significantly larger in bile-duct-ligated rats than in intact animals. These results suggest that the renal transport capacity for amphiphilic organic anions, such as PSP, might be increased compensatively in bile-duct-ligated animals, and that the apparent decrease in renal secretory transport for PSP might result from, at least in part, random distribution of the ligand to extrarenal tissues due to decrease in the binding activity of albumin.

Introduction

Transcellular transport in liver and kidney plays an important role in eliminating various amphiphilic metabolites from the organisms. Excretion of a group of organic anions with molecular weight of 300–700 occurs in both organs; excretion of these compounds by one route increases when the other is blocked [1,2]. Patients with liver diseases sometimes associate with dysfunction of extrahepatic tissues, such as the kidney [3,4]. The mechanism by which liver diseases often associate with renal dysfunction is not known. Although phenolsulfophthalein (PSP), a amphiphilic organic anion that circulates bound to albumin, is primarily a nephrophilic ligand, it is excreted by both organs in rodents. We previously showed that the rate of PSP excretion by the liver increased compensatively in nephrectomized normal rats but not in mutant Nagase analbuminemic rats (NAR) and suggested the critical importance of ligand–albumin interaction in the compensatory elimination of amphiphilic metabolites by the liver [5,6]. The present work examines the effect of bile-duct ligation on ligand binding activity of plasma protein(s) and renal transtubular secretion of PSP in the rat. Effect of bilirubin oxidase, an enzyme that selectively degrades amphiphilic bilirubin to water-soluble metabolites, on PSP binding of the jaundiced rat plasma was also studied. The results revealed that, although the renal transport capacity for an organic anion markedly increased in jaundiced rats, the transport system could not efficiently function predominantly due to decreased binding of a nephrophilic ligand to the circulating albumin, a biovehicle for amphiphilic organic anions [5–10].

Materials and Methods

Materials

PSP and rat serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO). Heparin was

Abbreviation: PSP, phenolsulfophthalein.

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purchased from Wako Pure Chemical Co. (Osaka). Bilirubin oxidase [EC 1.3.3.5 from *Myrothecium verricaria* MT-1] was obtained from Amano Pharmaceutical Co. (Nagoya).

Experiments in animals

Male Sprague-Dawley rats, 200–250 g, were fasted for 16 h prior to experiments. Under light ether anesthesia, the common bile duct was ligated just below the portal exit. Effect of bile-duct-ligation on renal transport function was studied 24 h after operation. Experiments for PSP transport were performed between 9:00 a.m. and 12:00 noon under pentobarbital anesthesia (50 mg/kg of body weight). Thirty min before each experiment, animals were heparinized intravenously (2500 unit/kg). PSP dissolved in 0.5 ml of saline was injected into the right femoral vein (10 μmol/kg) over a period of 5 s. At timed intervals, 0.1 ml of blood samples were collected from the right femoral vein and centrifugated at 12800 × *g* for 3 min in an Eppendorf 5412 centrifuge. Plasma PSP concentrations were determined spectrophotometorically at 557 nm in 0.1 M NaOH [5,6].

Urinary excretion of PSP

After 1 h of PSP administration, animals were exsanguinated by bleeding. Urine samples were collected by puncture of the urinary bladder and determined for PSP concentration. Then, a pair of kidneys were removed. The excised kidneys were homogenized in 3 vol. of 60% ethanol/2% perchloric acid (PCA) solution. The homogenates were centrifuged at 12800 × *g* for 3 min. Then, PSP concentrations in the acid soluble fraction were determined. Renal excretion of PSP was calculated as the sum of PSP found in the urine and the ethanol-extractable fraction of the homogenate.

Protein binding of PSP

Heparinized plasma samples were incubated with varying concentrations of PSP at 37°C for 10 min and centrifuged at 700 × *g* for 15 min in ultrafiltration cells using Amicon MPS-1 membranes. PSP concentrations in filtrates were determined spectrophotometrically at 557 nm as described previously [3].

Effect of bilirubin oxidase on PSP binding activity of plasma

Three days after bile-duct-ligation, plasma samples were obtained from jaundiced rats and incubated with bilirubin oxidase (0.3 unit/ml) at 37°C. After incubation for 1 h, PSP binding activity of plasma samples was determined by ultrafiltration method as described previously [11]. Various species of plasma bilirubin, such as mono- and diglucuronides were determined by high performance liquid chromatography [12].

Results

Fate of intravenously injected PSP in bile-duct-ligated animals

To study the effect of hepatic dysfunction on renal transport activity for organic anions, PSP was administered intravenously to intact and bile-duct-ligated animals (Fig. 1). Consistent with the previous observations [5], plasma PSP level decreased slowly in intact rats (half-life = 2.2 min). When PSP was injected to the bile-duct-ligated jaundiced rat, significant amount of the dye disappeared from the circulation almost instantaneously; plasma PSP level decreased thereafter with a half-life of 3.6 min. We previously reported that the rate of PSP disappearance from the circulation was significantly higher in NAR than in normal rats and that random distribution of its unbound fraction was responsible for the instantaneous decrease in plasma PSP levels in NAR [5]. Since biliary excretion of bilirubin and other cholephilic metabolites was blocked in bile-duct-ligated animals, their plamsa levels might be increased significantly and, hence, binding sites on albumin for such hydrophobic anions would be occupied in jaundiced rat. Thus, the instantaneous decrease in PSP level in jaundiced animals might reflect the increase in the unbound fraction of PSP. To test whether protein binding of PSP affects its transport, PSP was

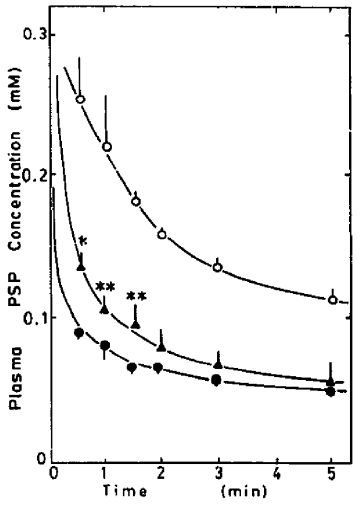

Fig. 1. Effect of bile-duct-ligation on plasma clearance of PSP. Under pentobarbital anesthesia, animals were intravenously injected with PSP (10 μmol/kg body weight). At the indicated times after administration, plasma levels of PSP were determined in control (○) and bile-duct-ligated animals (●). PSP was also injected with equimolar albumin in bile-duct-ligated animals (▲). Details of experimental conditions were described in the text. Each point represents mean value ± S.D. derived from three animals. * Significantly different from bile-duct-ligated animals which were injected with PSP without albumin (*$P < 0.01$, **$P < 0.05$).

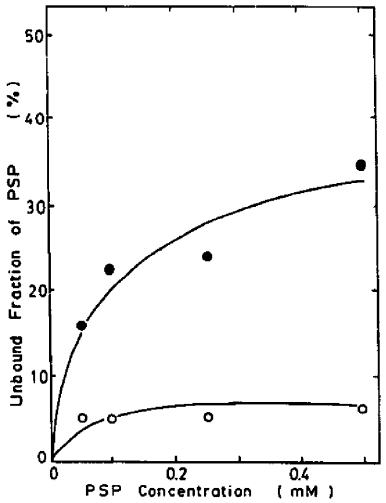

Fig. 2. Protein binding of PSP in intact and bile duct-ligated rat plasma. Plasma samples were obtained from intact (○) and bile-duct-ligated animals (●). After incubation of 0.5 ml of each plasma sample with varying concentrations of PSP at 37°C for 10 min, mixtures were centrifuged at 700 × g for 15 min in ultrafiltration cells. PSP concentrations in the filtrates were determined as described in the text. Each point shows a percentage of protein-unbound fraction of PSP.

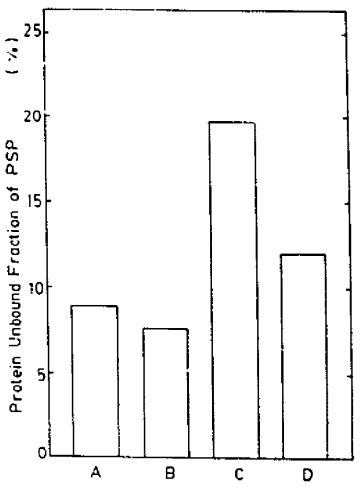

Fig. 3. Effect of albumin on PSP binding of normal and jaundiced rat plasma. Control and icteric plasma samples were obtained from intact (A, B) and bile-duct-ligated rats (C, D), respectively. After incubation of 0.5 ml of plasma with 0.1 mM PSP in the presence (B, D) or absence (A, C) of purified albumin (0.5 mM) at 37°C for 30 min, PSP binding activity of plasma samples was determined by ultrafiltration method as described in the text. Each result shows a percentage of protein-unbound fraction of PSP.

injected bound to equimolar albumin to bile-duct-ligated animals. The rate of PSP disappearance decreased significantly in jaundiced rats when PSP was injected bound to albumin. No appreciable effect of albumin on PSP clearance was found in intacts rats [5,6]. To know the effect of bile-duct-ligation on albumin function, PSP binding activity was compared with plasma samples from intact and bile-duct-ligated animals (Fig. 2). At PSP concentration lower than 0.5 mM, more than 93% of PSP bound to plasma protein(s) of intact rats. However, the unbound fraction of PSP markedly increased in jaundiced rat plasma. Addition of purified albumin to the jaundiced rat plasma markedly decreased the unbound fraction of PSP while no appreciable effect of albumin was found with intact rat plasma (Fig. 3), suggesting that the binding site(s) on albumin for PSP would be occupied by some metabolites. To know what metabolite(s) is responsible for the inhibition, PSP binding was determined before and after treating the jaundiced rat plasma with bilirubin oxidase. High performance liquid chromatographic analysis revealed that the major species of plasma bilirubin in bile-duct-ligated animals were accounted for by its di- and monoglucuronides; plasma level of unconjugated bilirubin was low (data not shown). When incubated with bilirubin oxidase, plasma bilirubin levels markedly decreased with time; bilirubin diglucuronide disappeared more rapidly than did monoglucuronide (Fig. 4). After degradation of bilirubin in bile-duct-ligated rat plasma, PSP binding capacity increased markedly (Fig. 5).

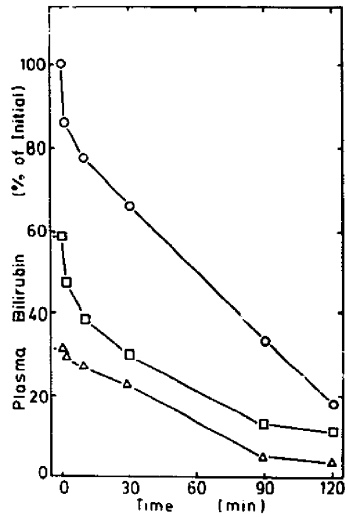

Fig. 4. Effect of bilirubin oxidase on plasma bilirubin levels. After incubation of icteric plasma obtained from bile-duct-ligated rats with bilirubin oxidase (0.1 unit/ml) at 37°C, plasma levels of various bilirubin species were determined by high performance liquid chromatography as described in the text. Each data shows a percentage of the initial level of each species. ○, total bilirubin; □, bilirubin diglucuronide; △, bilirubin monoglucuronide.

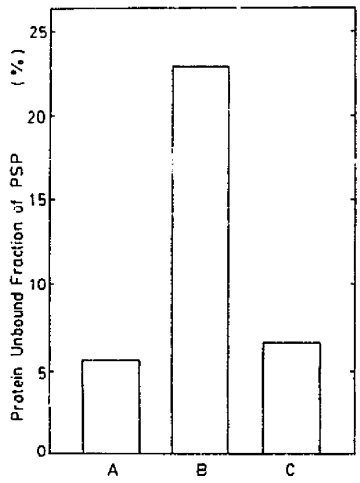

Fig. 5. Effect of bilirubin oxidase on PSP binding of icteric rat plasma. Plasma samples from bile-duct-ligated animals were incubated with bilirubin oxidase (3 unit/ml) at 37°C for 1 h. PSP binding activity of normal (A), icteric (B) and the oxidase-treated jaundiced plasma samples (C) was determined by ultrafiltration method as described in the text. Each result shows a percentage of protein-unbound fraction of PSP.

Effect of albumin on transtubular secretion of PSP in bile-duct-ligated rats

When injected intravenously, major part of PSP is excreted in urine in its unmetabolized form [13]. Tabel I shows the urinary excretion of PSP in normal and bile-duct-ligated animals. Urinary excretion of PSP markedly decreased in bile-duct-ligated animals. To know the effect of protein binding on transtubular transport for a nephrophilic ligand, PSP was injected bound to equimolar albumin to the intact and bile-duct-ligated animals. Albumin markedly enhanced the urinary secretion of PSP. It should be noted that the urinary recovery of PSP which was injected bound to albumin was significantly higher in bile-duct-ligated rats than when PSP was injected to intact animals. Administration of PSP with equimolar albumin had no appreciable effect on plasma clearance and urinary excretion of PSP in intact rats [5,6].

TABLE I

Urinary excretion of PSP

The common bile duct of the rat was ligated 24 h before experiments as described in the text. Under pentobarbital anesthesia, animals were intravenously injected with PSP (10 μmol/kg body weight). 60 min after administration of PSP with or without equimolar albumin, urinary excretion of PSP was determined in intact and bile-duct-ligated jaundiced rats as described in the text. Each value represents the mean ± S.D. for the urinary excretion of PSP (percent of dose) derived from three animals.

Experiments	Urinary excretion (% of dose)
Intact rats	30.7 ± 8.4
+ Albumin	32.0 ± 6.9
Jaundiced rats	15.5 ± 5.5
+ Albumin	56.2 ± 3.2

Discussion

The present work demonstrates that bile-duct ligation significantly increased PSP clearance and decreased plasma protein binding and urinary excretion of the nephrophilic ligand. Kinetic analysis using bilirubin oxidase revealed that bilirubin competed with PSP for the binding site on albumin in bile-duct-ligated rats and that the decreased ligand-albumin interaction might predominantly be responsible for the increased plasma clearance and decreased urinary recovery of the ligand in jaundiced animals. It should be noted that, when PSP was injected bound to albumin, urinary excretion of PSP increased significantly in bile-duct-ligated rats but not in intact animals; the urinary recovery was larger in the former (56% of the dose) than in the latter (31% of the dose). These observations suggested that renal transport capacity for organic anions might be increased in bile-duct-ligated animals.

It should be noted that, although albumin markedly decreased the unbound fraction of PSP in jaundiced plasma, the rate of PSP disappearance was normalized partially even if PSP was injected bound to albumin (see Fig. 1). Predominantly because of amphiphilic nature of bilirubin, when plasma bilirubin levels were increased markedly, it might bind not only to albumin but also to other plasma proteins, such as lipoproteins [8], and membrane/lipid bilayers of various tissues. Thus, a rapid exchange between PSP and bilirubin would occur on the injected albumin molecules in the circulation of jaundiced animals. We previously reported that intravenous administration of bilirubin oxidase transiently decreased the plasma bilirubin levels of bile-duct-ligated rats [14]. The decreased bilirubin level returned initial high level when the injected bilirubin oxidase disappeared from the circulation. It was also reported that, when albumin was infused intravenously in premature infants with hyperbilirubinemia, PSP binding capacity of the patients' serum increased with concomitant elevation of serum bilirubin levels [15]. These observations are concistent with the notions described above. These events may explain why PSP disappearance from the circulation of the jaundiced animals was normalized incompletely by administering the ligand with equimolar albumin.

It should be noted that a fairly large dose of albumin was injected in the present experiments. Administration of 10 μmol/kg of albumin would increase the plasma albumin level by about 150%. Such a marked increase in albumin level in the circulation may expand the plasma

volume and secondarily affect the excretory function of the kidney for a nephrophilic ligand. However, no appreciable effect of albumin loading on the urinary excretion of PSP was found in intact rats (Refs. 5,6, and Table I). Since administration of albumin-bound PSP markedly increased the urinary excretion of PSP in bile-duct-ligated rats, the expansion of plasma albumin pool may affect the circulatory status and renal transport functions of the animals under pathological conditions. This possibility should be studied further.

Since PSP is easily determined spectrophotometrically and shows no cytotoxicity, it has long been used clinically for renal function test. The present work suggested that plasma clearance and urinary excretion of PSP would be affected differently by some endogenous metabolite(s) with high affinity to albumin irrespective of the renal transport function for organic anions. Previous studies in analbuminemic rats also revealed that, despite a marked increase in PSP clearance, urinary excretion of the ligand was significantly lower than in normal subjects predominantly due to random distribution of the unbound PSP to extrarenal tissues [5]. Thus, the data obtained from renal function test using nephrophilic organic anions should be interpreted carefully to evaluate the renal status of a patient with liver diseases, such as obstructive jaundice.

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