GLUCURONIDATION OF 1-NAPHTHOL AND EXCRETION INTO THE VEIN IN PERFUSED RAT KIDNEY

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ABSTRACT:

UDP-glucuronosyltransferase is expressed in the proximal convoluted tubular cells of rat kidney. Kidney perfusion with a Krebs-Henseleit buffer containing 1-naphthol was performed to estimate the dynamics and disposition of the glucuronide conjugate formed in the epithelial cells of the renal tubules. When 1-naphthol was injected into the renal artery, and the perfusate from the renal vein was returned to a reservoir and recirculated through the kidney preparation (recirculating perfusion), most of the 1-naphthol was immediately excreted into the vein as a glucuronide conjugate and its concentration increased rapidly. In contrast, the 1-naphthol glucuronide appeared more slowly in the urine. 1-Naphthol was also injected during the initial 5 min of perfusion under single-pass perfusion conditions (single-pass perfusion) in situ, and the metabolite and parent compound in the venous perfusate and in urine were assayed. Under this condition, most of the 1-naphthol glucuronide was excreted into the renal vein, and not urine. Phenol UDP-glucuronosyltransferase was highly induced in the rat kidney by β-naphthoflavone treatment. Moreover, the amount of 1-naphthol glucuronide excreted in the renal vein was increased 2.7-fold in the perfused kidney of β-naphthoflavone-treated rats, but the amount in the urine was not significantly increased under single-pass perfusion conditions. These results indicate that the kidney can glucuronidate phenolic xenobiotics in epithelial cells of the tubules and excrete the resultant glucuronide into the renal vein.

Extensive oxidation, reduction, hydrolysis, and conjugation reactions of xenobiotics can occur in the kidney (Lohr et al., 1998). Moreover, renal drug metabolism can be a critical determinant of risk to chemical injury (Lash, 1994). Most drugs and environmental toxicants are eventually excreted into the urine as glucuronides. Kidney perfusion of drugs is a useful means for estimating the formation and excretion pathways of glucuronides. For example, after isolated kidney perfusion with 1-naphthol, most of the chemical was glucuronidated and excreted into the urine (Redegeld et al., 1988). Phenol UDP-glucuronosyltransferase, which glucuronidates various phenolic xenobiotics such as 1-naphthol, was shown to be expressed in epithelial cells of proximal convoluted tubules of normal rats and also in the cells of the distal tubules of β-naphthoflavone-treated rats (Yokota et al., 1997). Determination of the direction of efflux of the glucuronide formed in the cells is important to estimate the drug dynamics not only in the kidney but also in the whole body. The excretion direction depends on the locations of ATP-binding cassette (ABC) transporter members, such as multidrug resistance-associated protein (MRP), which transport drug-glucuronide out of the cells (Borst and Elferink, 2002). MRP1 and MRP3 are expressed at the basolateral membrane and MRP2 is expressed at the apical membrane in epithelial cells of renal tubules (Borst and Elferink, 2002). However, the direction of glucuronide transportation out of the cells is not known.

In this study, a 1-naphthol glucuronide, a major metabolite that is excreted into urine and veins from the perfused kidney, was analyzed, and most of the metabolite was found in veins as a glucuronide. The results indicate that excretion of 1-naphthol glucuronide out of epithelial cells of renal tubules occurs predominantly across the basolateral membrane.

Materials and Methods

Chemicals. Cholic acid, purchased from Nissui Yakuhin Co. (Tokyo, Japan), was further purified and converted to its sodium salt (Imai, 1979). UDP-glucuronic acid was obtained from Nakarai Yakuhin Co. (Kyoto, Japan). 1-Naphthol was purchased from Kanto Chemical Co. (Tokyo, Japan). High-performance liquid chromatography (HPLC)-grade acetonitrile was obtained from Labscan Ltd. (Dublin, Ireland). β-Glucuronidase was obtained from Sigma-Aldrich (St. Louis, MO). Other reagents were of the highest grade available.

Animals. Male Sprague-Dawley rats (8 weeks old, 250–280 g) were used in all experiments. The rats were housed under standard conditions and given food and water ad libitum before use. The rats were handled according to the Laboratory Animal Control Guidelines of Rakuno Gakuen University based on the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health in the United States.

Surgical Procedure and Kidney Perfusion. The rats were anesthetized by...
intraperitoneal injection of 60% urethane (0.3 ml/100 g body weight). Whole kidney perfusion was performed according to methods previously reported by Bowman (1975) and de Vries et al. (1989). Briefly, after anesthesia, the abdominal cavity was exposed by a ventral incision and the right ureter was cannulated with PE-10 polyethylene tubing (BD Biosciences, San Jose, CA), and then sodium heparin (1000 IU) was administered by an i.v. injection into the tail vein. Subsequently, the right renal artery was cannulated with a 21 gauge needle via the superior mesenteric artery. The vascular perfusate was a Krebs-Henseleit buffer containing 6% bovine serum albumin, 5 mM glucose, 2 mM glutamine, 2 mM glutathione, and 2 mM alanine. The buffer solution was aerated by 95% O₂ + 5% CO₂, and the pH was adjusted to 7.4. Several concentrations of substrate in the solution were tested, and full metabolites were obtained at 50 μM substrates in 30 ml of solution. These buffer solutions were maintained in a water bath at 37°C. The perfusion system consisted of a peristaltic pump (MP-32N, EYELA Co., Tokyo, Japan) and silicone tubes as illustrated in Fig. 1. The perfusion flow was maintained at 10 to 13 ml/min, and perfusion pressure was monitored.

Recirculating perfusion. The isolated kidney was fixed in a recirculating apparatus containing Krebs-Henseleit buffer (30 ml) as shown in Fig. 1. The buffer solution, which was mixed with the gas (95% O₂ + 5% CO₂), was perfused into the right renal artery, and the perfusate buffer yielded from the vein was returned to a reservoir and was recirculated through the kidney preparation (recirculating perfusion). The urine excreted out of the perfused kidney was collected as shown in Fig. 1 (final volume, 1.6 μl). An equilibration period of 15 min was conducted. Then 1-naphthol was added to the buffer solution (final concentration of 50 μM in 30 ml of solution). The substrate buffer solution was injected into the artery, and the resultant perfusate from the vein was mixed with the substrate buffer solution. Recirculation was continued for 40 min.

Single-pass perfusion. The perfusion was carried out in a flow-through mode. Preliminary perfusion was done for 15 min. Then, after a 5-min interval, the substrate buffer solution was perfused, and this was followed by re-perfusion of the Krebs-Henseleit buffer solution. After perfusion of the substrate buffer, the excreted urine and perfusat in the vein were sampled and assayed for metabolites by HPLC.

Viability. The viability of the perfused kidney was monitored by measuring glucose and albumin reabsorption (>95%), and by measuring creatinine clearance for glomerular filtration and p-aminohippuric acid clearance for tubular secretion. These parameters were all found to be within the expected normal ranges.

HPLC Analysis of Reaction Products. The perfusate and urine were filtered for removing proteins using Artkiss (Advantec Toyo Co., Tokyo, Japan). The filtered solution was stored at −80°C until analysis. Samples were analyzed by HPLC (Tosoh Co., Tokyo, Japan) according to the method described previously (Yokota et al., 1999; Daidoji et al., 2003).

β-Glucuronidase Reaction. For confirmation of 1-naphthol glucuronide, excreted metabolites were treated with β-glucuronidase using the method described previously (Yokota et al., 1999; Daidoji et al., 2003). The reaction was allowed to proceed for 30 min at 37°C in a mixture of 100 μl of 0.5 M acetate buffer (pH 4.5), β-glucuronidase (2.5 mg/ml), and diluted perfusate. The reaction mixture was then boiled and centrifuged for 5 min at 9000g. The supernatant was filtered through a disposable disk filter (HLC-DISK 3; Kanto Co., Tokyo, Japan) and analyzed by HPLC.

Treatment of Rats and Preparation of Microsomes. β-Naphthoflavone was dissolved in olive oil and orally administered at doses of 100 mg/kg of body weight daily for 4 days. The rats were killed 1 day after the final dose. The rat kidney was minced and homogenized with 4 vol of 0.15 M KCl solution containing 1 mM EDTA. The homogenate was centrifuged for 15 min at 9000g, and the supernatant fraction was centrifuged at 105,000g for 60 min to obtain microsomes.

Enzyme Analysis and HPLC. Renal microsomal UDP-glucuronosyltransferase activities toward 1-naphthol were assayed in 200 μl of 50 mM Tris-HCl buffer (pH 7.4), 5 mM UDP-glucuronic acid, 0.5 mM MgCl₂ containing 0.25 mM 1-naphthol at 37°C. The resultant enzyme reaction products were filtered through a disposable disk filter (HPLC-DISK 3; Kanto Co.) and analyzed by an HPLC system consisting of a Tosoh TSKgel 80TM reversed phase column (7.8 mm × 30 cm). The filtered samples were injected and eluted with an acetonitrile/H₂O/acetic acid (35:65:0.1 v/v) solution. 1-Naphthol glucuronide was determined by using authentic standards.

Results

Recirculation Perfusion. Several concentrations of substrate (1-naphthol) in the solution were tested in a pilot experiment, and quantitation time course data were obtained at a substrate concentration of 50 μM in 30 ml of solution. After injection of 1-naphthol into the rat renal artery with perfusion buffer over a period of 30 min, the concentrations of 1-naphthol glucuronide in the perfusate buffer and urine (Fig. 1) were determined by HPLC as described under Materials and Methods. 1-Naphthol glucuronide was detected immediately, and its concentration increased rapidly in the vein after recirculating perfusion (Fig. 2). However, glucuronide in the urine was excreted very slowly (Fig. 2). After 30 min of recirculation, the total amount of 1-naphthol glucuronide in the perfusate and in the urine was calculated to be 0.99 μmol (38 μM × 26 ml of perfusate, 66% of the total 1-naphthol dose), and 17.7 nmol (6.8 μM × 1.6 ml of urine, 0.007% of dose). These results suggested that 1-naphthol was glucuronidated in the proximal convolution tubular cells and was excreted into the renal vein, and that the glucuronide appeared in the urine only after filtration of recirculated perfusate in the kidney glomerulus. To test this hypothesis, a single-pass perfusion experiment was performed.

Single-Pass Perfusion. As shown in Fig. 3, the perfusion buffer was injected into the kidney artery in situ, and then the perfusate excreted from the renal vein was collected. The urine was also collected (single-pass perfusion), and the collected samples were analyzed by HPLC. 1-Naphthol or bisphenol A was injected into the renal artery over a period of only 5 min, and then the kidney was perfused with the buffer. The amounts of chemicals and their glucuronides were monitored, and the results are shown in Fig. 4. Large amounts of free 1-naphthol and the glucuronide were observed in the perfusate from the renal vein (80% yield) (Fig. 4A). Both were detected in the urine in very small amounts (Fig. 4A). Bisphenol A was detected only in the perfusate as unconjugated free compound (95% yield) (Fig. 4B). Renal microsomal UDP-glucuronosyltransferase activity toward 1-naphthol was induced about 3.4-fold by β-naphthoflavone treatment (Table 1) as previously described.

Fig. 1. Scheme of recirculating perfusion of the rat isolated kidney. The isolated kidney (k) was fixed in a tube containing Krebs-Henseleit buffer containing 6% bovine serum albumin, and the buffer solution (total, 30 ml) was pumped (p) through the kidney at 18 ml per min. The buffer solution was gassed with 95% O₂ + 5% CO₂ (g). The urine (final volume = 1.6 ml) was collected in a sample tube (l). a, artery; v, vein; u, ureter; r, reservoir.
1-Naphthol was perfused in kidneys from \(\text{H}9252\)-naphthoflavone-treated rats, and the results are shown in Fig. 5. After single-pass perfusion, the amount of 1-naphthol glucuronide excreted in to the vein was increased in the kidney of the rat treated with \(\text{H}9252\)-naphthoflavone; however, the glucuronide in the urine was not significantly affected by the treatment (Fig. 5). Total amounts of 1-naphthol glucuronide in the renal vein and urine after perfusion for 40 min are shown in Table 1. About 80.3% and 93.8% of the total amount of the glucuronide formed was excreted into the renal veins of control and \(\text{H}9252\)-naphthoflavone-treated rats, respectively (Table 1). The amounts collected from the renal veins of treated rats were about 2.8-fold greater than that from the veins of control rats (Table 1).

**Discussion**

UDP-glucuronosyltransferase, has been shown to be present in epithelial cells of proximal convoluted tubules of the rat kidney (Yokota et al., 1997). UGT1A6, an isofrom of the enzyme that glucuronidates phenolic substances such as 1-naphthol, but not
UGT2B1, an isoform that glucuronidates bisphenol A, was recently reported to be expressed in the rat kidney (Shelby et al., 2003). These findings agree with our results showing that formation and excretion of 1-naphthol glucuronide, but not bisphenol A glucuronide, was observed in the renal vein and urine from the kidney perfusion model. 1-Naphthol was principally metabolized to 1-naphthol glucuronide and excreted in large amounts into the urine following recirculating perfusion of the kidney (Redegeld et al., 1988; de Vries et al., 1989). We also obtained the same results with recirculating perfusion of the kidney (Fig. 2). In contrast, most of the 1-naphthol glucuronide was detected only in the renal vein after single-pass perfusion, suggesting renal formation and preferential excretion of the glucuronide across the basolateral membrane. However, it is possible that some of the glucuronide excreted into the tubular filtrate side may be reabsorbed by tubular epithelial cells and diffuse into the renal vein. UDP-glucuronosyltransferase was induced and detected in the epithelial cells not only of the proximal convoluted tubules but also of the straight portion of the distal tubules after β-naphthoflavone treatment of rats (Yokota et al., 1997). The amount of the resultant glucuronide formed in the perfused kidney was increased in proportion to the enzyme activity of renal microsomal UDP-glucuronosyltransferase in β-naphthoflavone-treated rats. An additional cause of an increase in 1-naphthol glucuronide in the renal vein was thought to be induction of the transporters mediating the glucuronide transport. These results indicate that 1-naphthol glucuronides are transported into the renal vein from epithelial cells of proximal and distal tubules.

The membrane proteins belonging to the ABC transporter family play an important role in the excretion of many substrates and metabolites from cells (Borst and Elferink, 2002). Members of the ABC family of MRPs, such as MRP1, MRP2, and MRP3, accept glucuronide conjugates. In the liver, MRP2 mediates the efficient biliary excretion of glucuronides and is expressed on the bile canalicular membrane (Borst and Elferink, 2002). MRP2 is expressed not only in the liver but also in the kidney, where it is located in the apical membrane of epithelial cells (Schaub et al., 1997). Significant disturbance of renal clearance of 1-naphthol glucuronide was found in isolated perfused kidneys of TR transport-deficient rats, which are deficient in MRP2 (de Vries et al., 1989), indicating that 1-naphthol glucuronide is a substrate of MRP2. Thus, MRP2 could be responsible for the secretion of 1-naphthol glucuronide into the urine (13–20% of total glucuronide). MRP1 and MRP3 are expressed at basolateral membranes of distal convoluted tubular epithelial cells (Kool et al., 1999; Van Aubel et al., 2000; Scheffer et al., 2002), but neither of them appears to be expressed in the proximal tubule (Peng et al., 1999). MRP1 and MRP3 are thought to transport 1-naphthol glucuronide across the basolateral membranes of distal convoluted tubular epithelial cells into the renal vein in β-naphthoflavone-treated rats, but the transporter in proximal tubular epithelial cells in which UDP-glucuronosyltransferase is expressed under normal conditions is unknown. Since the direction and speed of glucuronide excretion depend on the location and properties of the transporter in epithelial cells, it is important to identify the transporter.

Based on the results described above, it is concluded that 1-naphthol injected into the renal artery is absorbed into epithelial cells and glucuronidated. The resultant glucuronide is transported primarily across the basolateral membrane into the vein and circulates through the whole body. The glucuronide is then filtrated through the glomerulus of the kidney into urine.

References

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