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Glucuronidation and the Transport of the Glucuronide Metabolites in LLC-PK1 Cells

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Abstract: Formation and transport of glucuronide metabolites were studied in LLC-PK1 cells. Glucuronidation of 17β-estradiol, 1-naphthol, mycophenolic acid, and 4-methylumbelliferone was examined in microsomes prepared from LLC-PK1 cells, human livers, human kidneys, and human intestines. The rate of glucuronide metabolite formation observed with LLC-PK1 microsomes was comparable to rates observed with various human tissue microsomes. The fate of the glucuronide metabolite formed in the LLC-PK1 cells was studied by examining its extracellular transport using mycophenolic acid as a model substrate. After administration of mycophenolic acid, the amount of the glucuronide metabolite exiting to the extracellular compartments significantly decreased in the presence of MK-571, an inhibitor for the multidrug resistance-associated protein (MRP) transporter. However, the intracellular levels of the glucuronide metabolite did not change, suggesting that MK-571 was probably blocking metabolite efflux. In summary, these results suggest that the glucuronidating enzyme(s) expressed in the LLC-PK1 cells are capable of sufficient glucuronidation activity and that endogenous transporter(s) in LLC-PK1 cells are active and determine the distribution of the formed metabolites. Since these cells have been previously used to study drug transport, they may be a useful tool in future studies to explore the effect of drug transporters on glucuronidation.

Keywords: Glucuronidation; LLC-PK1; UDP-glucuronosyltransferase

Mammalian epithelial cell lines such as the LLC-PK1 cells are useful tools to characterize a variety of drug transporters and to examine the transporter affinities of many compounds.[1−4] Because they are easy to culture and maintain compared to previous methods such as Ussing chambers and intestinal perfusions, these epithelial cells have been commonly employed to examine drug transport. LLC-PK1 is a porcine kidney epithelial cell line that like the human intestinal epithelium expresses microvilli and tight junctions.[5] LLC-PK1 cells share many common drug transporters as found in the intestine and correctly distribute them to the appropriate membrane, such as multidrug resistance-associated protein-1 (MRP1) to the serosal membrane and MRP2 and multidrug resistance protein (MDR1) to the luminal membrane, to form a polarized epithelial.[6,7] Although it is

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not a human cell line, lack of significant phase I enzyme activities[8] and relative success of transfecting with human drug transporter(s) have allowed LLC-PK1 cells to be widely used to examine transporter function. In fact, studies have shown that P-glycoprotein (Pgp) transfected LLC-PK1 showed a strong correlation to Pgp function determined by intestinal perfusion models.[9] Recently however, LLC-PK1 cell lines have been shown to possess glucuronidation activity.[10] Therefore, the ability to pass through these cells may depend not only on drug transporters but also on metabolism for compounds whose main metabolic pathway may be glucuronidation.

Glucuronidation is a phase II biotransformation reaction catalyzed by UDP-glucuronosyltransferases (UGTs). These enzymes are involved in the conjugation of mainly lipophilic compounds with glucuronic acid via a S_N2 mechanism.[11] The resulting metabolites are water-soluble β-D-glucuronides that can be readily eliminated from the body via the urine or into the bile. MRP2 has been shown to help mediate these processes.[12] Although the liver is an important organ involved in glucuronidation, recent evidence has shown that extrahepatic organs such as the kidney and the intestine may also be significant. The expressions of select UGT1A isoforms in rat kidney and intestines have been shown to be as much as (if not greater than) those in the rat liver.[13]

In human, nine of the 16 UGT isoforms currently identified are encoded by the UGT1A locus while seven UGTs are encoded by the UGT2B locus.[14,15] Of the 9 UGT1A isoforms, UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9 have been shown to be expressed in the liver; UGT1A8 and UGT1A9 in the kidney; and UGT1A1, UGT1A4, UGT1A8, and UGT1A10 in the intestine.[14,16] However, there are discrepancies. For example, Cheng et al.[17] reported that UGT1A8 was expressed in the human ileum, but later work by Strassburg et al.[18] failed to detect the expression of UGT1A8 in 5 human ileum samples. Similar discrepancies were demonstrated with UGT1A5[15,18] and UGT1A6.[11,19]

In the present study, the intrinsic clearance (CL_{int}) of drug glucuronidation was examined in LLC-PK1 cells using several substrates. Since there are no set criteria to classify enzyme activity as significant or negligible, the CL_{int} values of glucuronidation in LLC-PK1 cells were compared with those observed in several human tissues. Furthermore, because the inherent properties of the metabolites make them impermeable toward biological membranes via passive diffusion, the role of endogenous transporter(s) to move the glucuronide metabolites out of the LLC-PK1 cells was examined by monitoring the extracellular flux of the formed metabolites in the presence of an MRP inhibitor, MK-571.

Materials and Methods

Materials. 17β-Estradiol (17ES), 1-naphthol (1NP), mycophenolic acid (MPA), 4-methylumbelliferone (4MU), 17β-estradiol β-glucuronide (17ESG), 1-naphthol glucuronide (1NPG), mycophenolic acid glucuronide (MPAG), 4-methylumbelliferone glucuronide (4MUG), Brij-58, UDP-glucuronic acid (UDPGA), phenylmethylsulfonyl fluoride (PMSF), ethylenediaminetetraacetic acid (EDTA), dithiothreitol (DTT), Tris, sucrose, potassium chloride, potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and glycerol were purchased from Sigma Aldrich (St. Louis, MO). Saccharolactone was purchased from Aldrich Chemical Company (Milwaukee, WI). MK-571 was purchased from BIOMOL Research Laboratories (Plymouth Meeting, PA). Tacrolimus was obtained from Fujisawa Pharmaceutical Co. (Osaka, Japan).

Human liver microsomes (pool of 4 donors from specimens HG3, HG42, HG56, and HG89) were purchased from

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BD Biosciences (Woburn, MA). Pooled human kidney (from 6 donors) and human intestinal microsomes (from 10 donors) were purchased from In Vitro Technologies (Baltimore, MD).

LLC-PK1 cells were a generous gift from Prof. Piet Borst (The Netherlands Cancer Institute; Amsterdam, The Netherlands). M-199 with Earle's BSS medium containing 1.0 g/L glucose, 0.1 g/L L-glutamine, and 2.2 g/L $NaHCO_3$; and Hanks BBS without phenol red containing calcium and magnesium salts, 0.4 g/L KCl, 0.06 g/L KH_2PO_4, 8.0 g/L NaCl, 1.0 g/L glucose, 0.09 g/L $Na_2HPO_4 \cdot 7H_2O$, and 0.35 g/L $NaHCO_3$ were custom-made at the UCSF Cell Culture Facility (San Francisco, CA). Fetal bovine serum (FBS) was purchased from HyClone Laboratories (Logan, UT).

LLC-PK1 Culture Conditions. All cells were incubated at 37 °C under a 5% humidified CO_2 atmosphere. LLC-PK1 cells (passage number $P + 16$ to $P + 20$) were cultured in M-199 medium containing 10% FBS. The growth medium was replaced three times prior to harvesting of the cells. The medium was replaced the day after the cells were split (seed day + 1) followed by seed day + 3 and seed day + 5. The cells were harvested on seed day + 6.

Preparation of LLC-PK1 Microsomes. LLC-PK1 cells (1.5−2 million) were seeded on 100 × 15 mm Petri dishes. The cells were cultured as described above. The cells were harvested when they reached confluence. Briefly, the medium was removed, and the cells were washed with 10 mL of 0.15 M KCl. Using a cell scraper, the cells were scraped off the bottom of the Petri dish in 5 mL of homogenizing buffer (50 mM Tris HCl pH 7.4, 1 mM EDTA, 1 mM DTT, 0.1 mM PMSF, 0.15 M KCl, and 0.25 M sucrose). The harvested cells were homogenized on ice utilizing a glass homogenizer from Wheaton Science Products (Millville, NJ). The homogenized cells were then centrifuged at 2000*g* for 20 min at 4 °C. The supernatant was collected and centrifuged at 12000*g* for 20 min at 4 °C. This supernatant was collected and centrifuged at 100000*g* for 65 min at 4 °C. The pellet was collected and resuspended in washing buffer (0.15 M KCl, 1 mM EDTA, and 0.1 mM PMSF) using a hand driven Teflon-glass homogenizer. The resuspended fraction was centrifuged at 100000*g* for 65 min at 4 °C. The final microsomal pellet was resuspended in storage buffer (0.1 M potassium phosphate pH 7.4, 1 mM EDTA, 1 mM DTT, and 20% glycerol) utilizing the hand driven Teflon-glass homogenizer. Protein concentrations were determined with the Bio-Rad protein assay kit using bovine serium albumin (BSA) as a standard. 145−200 million cells yielded about 7−10 mg of microsomal protein. The microsomes were stored at −80 °C.

Glucuronidation Assays. Kinetic studies utilizing pooled microsomes were performed as described by Fisher et al.[20] with slight variations. Briefly, microsomes (0.5 mg/mL protein concentration in the incubation), Brij-58 (substrate dependent as listed below), and 0.1 M phosphate buffer (pH 7.1) were mixed and chilled on ice for 15 min. Preliminary experiments indicated that the optimal Brij-58 concentrations in incubations were 0.05 mg/mL, 0.1 mg/mL, 0.025 mg/mL, and 0.025 mg/mL for 17ES, 1NP, MPA, and 4MU, respectively. $MgCl_2$ (1 mM incubation concentration), saccharolactone (5 mM incubation concentration), and the substrate (10−250 μM for 17ES and 1NP; 0.5−80 μM for MPA; and 25−500 μM for 4MU) were mixed and incubated at 37 °C. After a 5 min preincubation, the reaction was started with UDPGA (5 mM incubation concentration). The total incubation volume was 250 μL. Preliminary experiments indicated that metabolite formation for all compounds was linear at least up to 30 min. Therefore, all reactions were stopped with 250 μL of ice-cold acetonitrile at 30 min. Each sample was then centrifuged to collect the supernatant. All samples were run with a negative control that consisted of the reaction mixture with no UDPGA. All experiments were done in triplicate, and the reported values represent the mean ± SD.

Transcellular Transport Study. MPAG transport was examined utilizing the protocol outlined by Cummins et al.[21] with minor modification. Briefly, cells growing on PET (polyethylene terephthalate) inserts were washed with transport buffer (Hanks BBS solution containing 25 mM HEPES and 1% FBS at pH = 7.4) for 20−30 min at 37 °C. The transepithelial electrical resistance (TEER) values were measured across the cell monolayers utilizing the Millipore Millicell (Bedford, MA) equipped with chopstick electrode probes. All TEER values were >220 $\Omega \cdot cm^2$. The "donor" solution contained 100 μM MPA (0.25% methanol) with or without 50 μM MK-571 (<0.01% DMSO of total volume) dissolved in transport buffer. The "receiver" solution contained 0.25% methanol with or without 50 μM MK-571 (<0.01% DMSO of total volume) dissolved in transport buffer. The experiment was initiated by adding the receiver solution to the basolateral compartment and the donor solution at the apical compartment for A → B transport. The receiver solution was added to the apical compartment and the donor solution to the basolateral compartment for B → A transport. The final volumes in the apical and basolateral compartments were 1.5 mL and 2.5 mL, respectively. At 3 h, 100 μL of receiver solution was sampled. The sample was diluted with 100 μL of 10−20 μM tamoxifen, the internal standard. The samples were analyzed for MPAG using LC/MS as described below. All experiments were done in triplicate.

LC/MS Methods. The glucuronide metabolites were quantified on the LC/MS (Agilent Technologies, Palo Alto, CA) utilizing the column switching system outlined by Christians et al.[22] with some modifications. This column switching method prevented impurities from entering the LC/MS system and allowed for a shorter LC gradient. Briefly,

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50 μL of sample was injected onto the LC/MS and loaded onto a Hypersil MOS extraction column (20 × 4 mm; 5 μm; Agilent Technologies, Palo Alto, CA), where it was washed with 2 mM ammonium acetate at a flow rate of 5 mL/min. After 1 min of washing, the switching system backflushed the samples from the extraction column onto a Phenomenex Prodigy column (50 × 2 mm ODS; 5 μm; Phenomenex, Torrance, CA), where the gradients listed below for each compound and its glucuronide metabolite were run. At the completion of the gradients, the switching column flushed into the extraction column to clean and reequilibrate to initial conditions.

A gradient was used for all analyses. The mobile phase consisted of 2 mM ammonium acetate and acetonitrile. For 17ES, 1NP, and MPA and their corresponding metabolites, the following gradient was run at 0.5 mL/min: 5% acetonitrile for 4.3 min; 5% to 95% acetonitrile for 2.2 min; and 95% acetonitrile for 1.5 min. For 4MU, the following gradient was run at 0.5 mL/min: 4% to 10% acetonitrile for 2.5 min; 10% to 65% acetonitrile for 2.5 min; 35% to 80% acetonitrile for 0.5 min; and 4% acetonitrile for 2 min. Negative ion mode gave the best detection for 17ES, 1NP, and MPA and their glucuronide metabolites, while positive ion mode gave the best detection for 4MU and its glucuronide metabolite: m/z = 271.3 and 447.3 for 17ES and 17ESG, respectively; m/z = 143.1 and 319.1 for 1NP and 1NPG, respectively; m/z = 271.2 and 447.3 for MPA and MPAG, respectively; and m/z = 177.1 and 353.1 for 4MU and 4MUG, respectively.

Data Analysis. Winnonlin Professional Edition version 2.1 from Pharsight Corporation (Palo Alto, CA) was utilized to determine the kinetic parameters of metabolite formation for 17ES, 1NP, MPA, and 4MU. K_m and V_{max} values were generated using "Compiled Pharmacodynamic" model ($effect_{0-inf}$), and these values were utilized to calculate the intrinsic clearance (CL_{int}) defined as

$$CL_{int} = V_{max}/K_m$$

Results and Discussion

Metabolite formation kinetics for 17β-estradiol (17ES), 1-naphthol (1NP), mycophenolic acid (MPA), and 4-methylumbelliferone (4MU) were determined in microsomes prepared from LLC-PK1 cells (LPM), human liver (HLM), human kidney (HKM), and human intestines (HIM). These compounds were chosen because they are directly glucuronidated without requiring phase I metabolism and because glucuronidation is the major metabolic pathway. It has been shown that one major route of metabolism for 17ES is

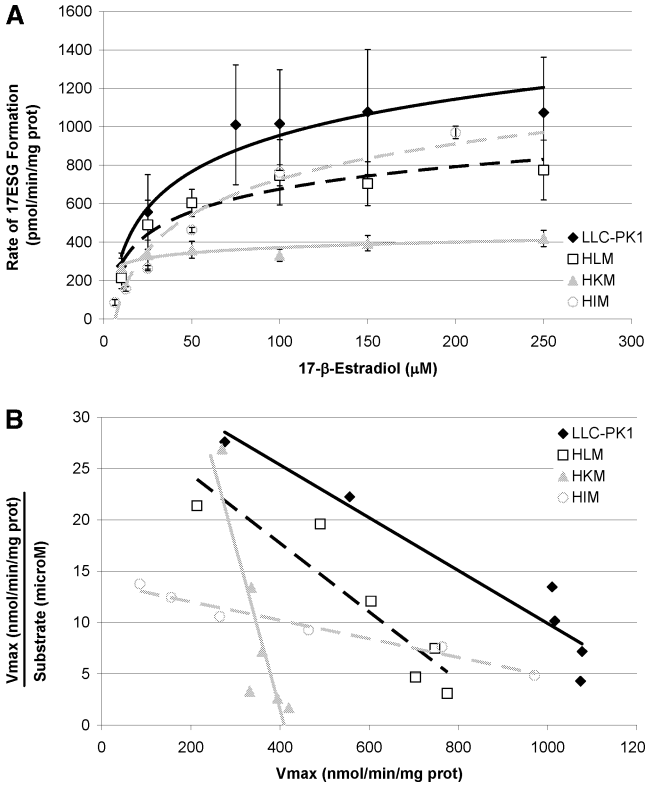

Figure 1. 17β-Estradiol β-glucuronide formation in LPM, HLM, HKM, and HIM shown as (A) Michaelis−Menten plot and (B) representative Eadie−Hofstee plot. Values for the Michaelis−Menten plot represent the mean of 3 experiments ± SD.

glucuronidation at the 17 position[23,24] and that this process is mediated by UGT1A3, UGT1A4, and UGT2B7.[25] MPA is primarily converted to the 4-*O*-glucuronide metabolite[26] mediated by UGT1A1, UGT1A7, UGT1A8, UGT1A9, and UGT1A10.[27] 4MU and 1NP are known to be extensively metabolized and oftentimes have been employed as probe substrates for human UGT1A6.[28,29]

The rates of metabolite formation at 30 min for a range of incubation concentrations in LPM reached saturation for

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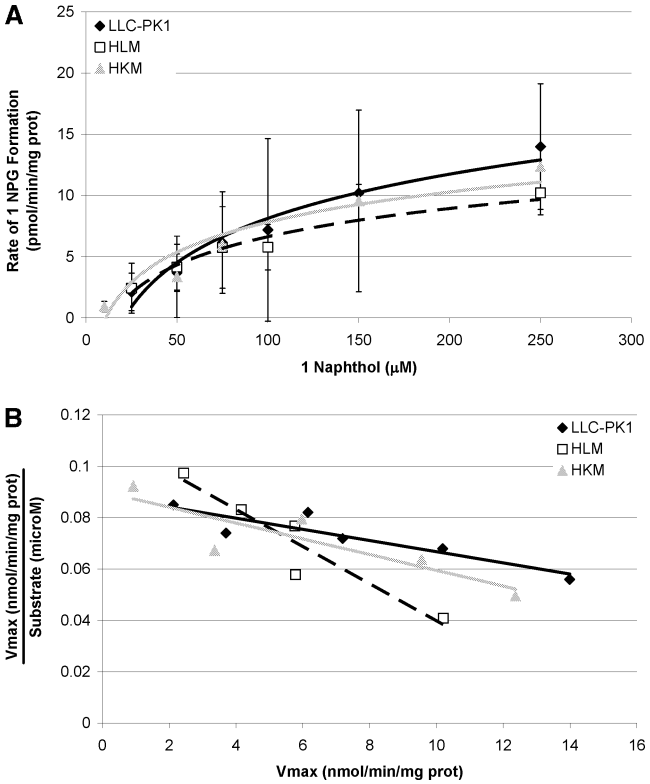

Figure 2. 1-Naphthol glucuronide formation in LPM, HLM, and HKM shown as (A) Michaelis−Menten plot and (B) representative Eadie−Hofstee plot. Values for the Michaelis−Menten plot represent the mean of 3 experiments ± SD.

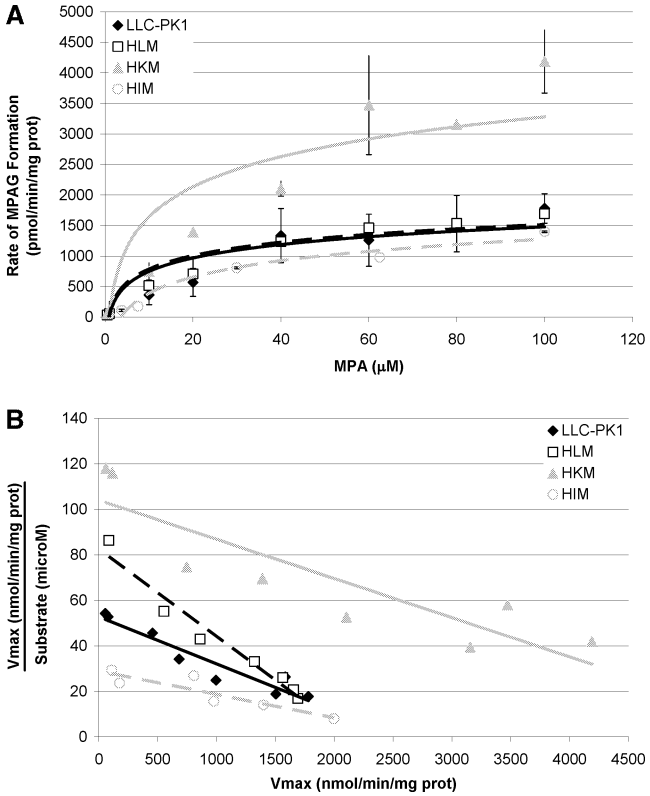

Figure 3. Mycophenolic acid glucuronide formation in LPM, HLM, HKM, and HIM shown as (A) Michaelis−Menten plot and (B) representative Eadie−Hofstee plot. Values for the Michaelis−Menten plot represent the mean of 3 experiments ± SD.

all compounds examined (Figures 1−4). When the Michaelis−Menten plots were converted to Eadie−Hofstee plots, the glucuronidation of the 4 substrates was linear (Figures 1−4). The linearity however, may not necessarily suggest that only one enzyme is responsible for the metabolism. This may be especially true for compounds undergoing glucuronidation since the isoform specificity of UGTs is broad. There are several reasons why contributions from multiple enzymes may go unnoticed. One reason may be that the K_m and V_{max} values are similar. Kinetic parameters for UGT1A6 and UGT1A9 in 1NP glucuronidation were found to be similar.[30] Similarly, if two enzymes have the same K_m but considerably different V_{max}, the kinetics may seem linear with $V_{max} = V_{max1} + V_{max2}$. Another reason may be that, between two enzymes, the contributions from the enzyme with the higher K_m and lower V_{max} may be obscured.

Kinetic parameters (K_m and V_{max}) for the rate of metabolite formation based on simple Michaelis−Menten kinetics were generated using Winnonlin and are summarized in Table 1. Despite some similarities in the kinetic parameters among the several matrices for the various substrates, there were some differences. For example, the K_m values for 17β-estradiol showed significant variability between LPM, HKM, and HIM. Since kinetic values were obtained in separate species and tissues, these discrepancies are not surprising. Dissimilarity may be attributed to species selectivity of the UGT enzymes. In addition, the differences in the composition of UGT isoforms in the kidneys and the intestine may also contribute to this variability.

Since the aim of this work to show that significant glucuronidation activity in LLC-PK1 cells was more quantitative than qualitative, the intrinsic clearance (CL_{int}) was examined. CL_{int}, or V_{max}/K_m, measures enzyme efficiency and has been frequently employed to compare enzymes by examining metabolic stability. Because there is no criterion that distinguishes between enzyme activity as significant or insignificant, the CL_{int} values from several human tissue microsomes were employed as a gauge of glucuronidation. The CL_{int} data in Table 1 suggest that glucuronidation in LPM is comparable to that in microsomes prepared from

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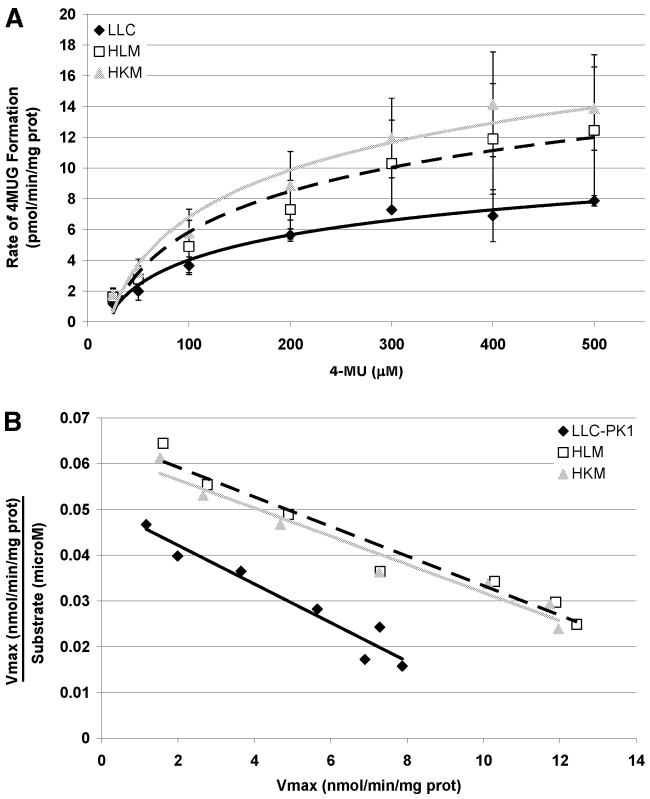

Figure 4. 4-Methylumbelliferone glucuronide formation in LPM, HLM, and HIM shown as (A) Michaelis−Menten plot and (B) representative Eadie−Hofstee plot. Values for the Michaelis−Menten plot represent the mean of 3 experiments ± SD.

various human tissues (at 0.5 mg/mL protein concentration), suggesting that drug glucuronidation in LLC-PK1 cells is significant. This is the first report showing that glucuronidation of porcine UGT enzymes against a variety of compounds in LLC-PK1 cells is significant. These findings imply that multiple components (i.e., drug transporters along with drug glucuronidation) may affect the transcellular movement of a drug molecule whose main metabolic pathway is glucuronidation.

To determine the fate of the metabolites produced within the LLC-PK1 cells, transport of a representative formed glucuronide metabolite was examined. After 100 μM MPA was loaded to the apical or the basolateral donor compartments, the efflux of MPAG was monitored. Within the 3 h incubation, MPAG was able to cross the plasma membrane to exit the cells and to enter the extracellular receiver compartment (Figure 5). Anionic conjugates cannot effectively exit the cell without active transporters such as MRPs.[12] Therefore, to investigate if endogenous MRP transporter(s) was involved in the transport of MPAG, its transport was monitored in the presence of an MRP inhibitor, MK-571. In the presence of 50 μM MK-571, the amount of MPAG found in the extracellular compartment was significantly lower than in the control without MK-571 (Figure 5A), suggesting that MRP-like homologue(s) may be active and involved in the extrusion of the metabolites from the cell. It has been previously shown that MK-571 may also inhibit glucuronidation,[31] and therefore, the change may be a result of enzyme inhibition rather than transport inhibition. However, when the intracellular levels were examined in LLC-PK1 cells, MK-571 did not change the amount of MPAG (Figure 5B), suggesting that although MK-571 may effect glucuronidation, the differences in MPAG efflux is probably a transporter effect.

The coupling of UGT and glucuronide efflux transporters was recently examined with raloxifene as a model substrate.[32] Here, the authors showed that active glucuronide transporters are directly coupled to glucuronidating enzymes to influence the dynamics of parent−glucuronide metabolite equilibrium

Table 1. Summary of Glucuronidation Kinetics in Various Microsomal Matrices[a]

matrix	K_m (μM)	V_{max} [nmol min^{-1} (mg of prot)$^{-1}$]	V_{max}/K_m [μL min^{-1} (mg of prot)$^{-1}$]
		17β-Estradiol	
LPM	19.9 ± 4.2	1.20 ± 0.30	60.3 ± 19.7
HLM	16.7 ± 3.3	0.81 ± 0.08	48 ± 11 (53[b])
HKM	5.1 ± 2.4	0.40 ± 0.03	78 ± 37
HIM	41.3 ± 0.3	1.26 ± 0.05	30.5 ± 1.2
		1-Naphthol	
LPM	132 ± 45	15.5 ± 2.9	117 ± 46
HLM	216 ± 99	20.2 ± 0.2	93.5 ± 42.9 (117[c])
HKM	138 ± 35	14.4 ± 4.2	104 ± 40
HIM	na	na	104 ± 65
		Mycophenolic Acid	
LPM	49.5 ± 13.1	2.10 ± 0.67	42.4 ± 17.6
HLM	37.6 ± 12.9	2.20 ± 0.15	58.5 ± 20.5 (46.6[d])
HKM	69.3 ± 29.0	6.00 ± 1.75	86.6 ± 44.2
HIM	114 ± 51	3.00 ± 0.26	26.3 ± 12.0
		4-Methylumbelliferone	
LPM	201 ± 45	11.1 ± 0.1	55.2 ± 12.4
HLM	350 ± 88	22.1 ± 9.8	63.1 ± 32.2 (30[e])
HKM	359 ± 75	21.1 ± 3.4	58.8 ± 15.5
HIM	nd	nd	nd

[a] K_m, V_{max}, and intrinsic clearance (V_{max}/K_m) values are listed for 17β-estradiol, 1-naphthol, mycophenolic acid, and 4-methylumbelliferone glucuronidation in LLC-PK1 microsomes (LPM), human liver microsomes (HLM), human kidney microsomes (HKM), and human intestinal microsomes (HIM). Values represent the mean of 3 replications ± SD; nd, not determined; na, not applicable. At the 1-naphthol concentrations tested, saturation was not observed but V_{max}/K_m was estimated from initial slopes. [b] Literature value reported as a mean of 3 determinations from 4 human livers.[20] [c] Literature value reported as a mean of 12 spearate incubations from 6 human livers.[39] [d] Literature value reported as mean of 5 determinations from 5 human livers.[40] [e] Literature value reported mean of 6 determinations from 1 human female liver.[41]

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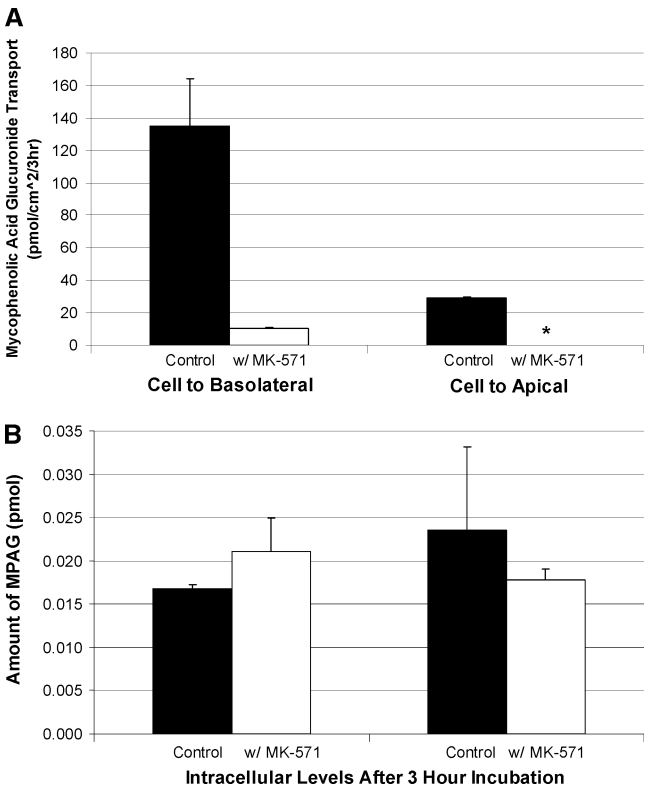

Figure 5. Amount of MPAG found at the (A) extracellular compartments and (B) intraceullar compartment in the LLC-PK1 cells. Amount of MPAG was measured in the absence (solid bar) or presence of 50 μM MK-571 (open bar) after 3 h. MPA (100 μM) was administered to the apical (cell to basolateral) or basolateral (cell to apical) compartment. "Cell" represents the source of MPAG, which is the LLC-PK1 cells. Values represent the mean of 3 experiments ± SD. There were no statistical differences in the intracellular levels of MPAG. (*) MPAG was not detected at this point.

present within the Caco-2 cells. In the present study, the intracellular levels were unchanged despite the significant decrease of MPAG transport to the extracellular compartment in the presence of MK-571, suggesting that, between the formation and exit of MPAG from the LLC-PK1 cells (i.e., metabolism and transport of MPAG), MPAG exit is the slower process. Therefore, in such situations, blocking MPAG efflux may have minimal effect on the parent−glucuronide equilibrium.

Mammalian cell lines have been increasingly utilized to identify compound specificities against various transporters and to characterize drug transporters. Cell lines such as LLC-PK1 cells were employed with the idea that contributions from drug metabolizing enzymes were minimal.[8] However, this study showed that LLC-PK1 cells are capable of significant glucuronidation, implying that functional data involving extensively glucuronidated compounds should be interpreted more carefully. Although information regarding the porcine UGT genetics is still unknown, it may be worthwhile in the future to examine what porcine UGT isoforms are expressed in LLC-PK1 and how they compare to human UGTs. Recently, it has been hypothesized that the activities of transporters and metabolizing enzymes are coupled.[2,21,33−38] These new findings suggest that LLC-PK1 may provide a useful model to study the possible relationship between glucuronidation and drug transporters.

Abbreviations Used

UGT, UDP-glucuronosyltransferase; MDR1, multidrug resistance protein; MRP, multidrug resistance-associated protein; UDPGA, UDP-glucuronic acid; LC/MS, liquid chromatography and mass spectrometry; LPM, LLC-PK1 microsomes; HLM, human liver microsomes; HKM, human kidney microsomes; HIM, human intestinal microsomes; 17ES, 17β-estradiol; 17ESG, 17β-estradiol β-glucuronide; 1NP, 1-naphthol; 1NPG, 1-naphthol glucuronide; MPA, mycophenolic acid; MPAG, mycophenolic acid glucuronide; 4MU, 4-methylumbelliferone; 4MUG, 4-methylumbelliferone glucuronide.

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