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Research Paper

Differential disposition of intra-renal generated and preformed glucuronides: studies with 4-methylumbelliferone and 4-methylumbelliferyl glucuronide in the filtering and nonfiltering isolated perfused rat kidney

Jiping Wang[a], Allan M. Evans[a], Kathleen M. Knights[b] and John O. Miners[b]

[a]Sansom Institute, School of Pharmacy and Medical Sciences, University of South Australia, and [b]Department of Clinical Pharmacology, Flinders University School of Medicine, Flinders Medical Centre, Adelaide, SA, Australia

Abstract

Objectives This study was designed to investigate the renal disposition of 4-methylumbelliferone (4MU) and 4-methylumbelliferyl glucuronide (4MUG) to characterise the contribution of excretion and metabolic clearance to total clearance in the kidney.
Methods The isolated perfused kidney (IPK) from the male Sprague–Dawley rat was used in filtering and non-filtering mode to study the renal disposition of 4MU, renally generated 4MUG and preformed 4MUG. Perfusate and urine (filtering IPK only) was collected for up to 120 min and 4MU and 4MUG in perfusate and urine were determined by HPLC. Analytes were also measured in kidney tissue collected at 120 min. Non-compartmental analysis was used to derive pharmacokinetic parameters.
Key findings The concentration of 4MU in perfusate declined with a terminal half-life of approximately 120 min following administration to the filtering IPK and nonfiltering IPK. There was a corresponding increase in the concentration of 4MUG. Metabolic clearance of 4MU accounted for 92% of total renal clearance. After bolus dosing of preformed 4MUG in the perfusion reservoir of the filtering IPK, the perfusate concentration declined with the terminal half-life of approximately 260 min. The renal excretory clearance of preformed 4MUG accounted for 96% of total renal clearance. 4MU was extensively metabolized by glucuronidation in the filtering and nonfiltering IPK, and the total renal clearance of 4MU was far greater than its renal excretory clearance. This indicated that glucuronidation was the major elimination pathway for 4MU in the kidney.
Conclusions The data confirmed an important role for the kidney in the metabolic clearance of xenobiotics via glucuronidation and signalled the lack of impact of impaired glomerular filtration on renal drug metabolism.
Keywords glucuronidation; isolated perfused rat kidney; 4-methylumbelliferone; pharmacokinetics; preformed glucuronide

Introduction

Glucuronidation represents both a clearance and detoxification mechanism for a myriad of chemicals, including drugs, nondrug xenobiotics and endogenous compounds.[1,2] Being charged and polar at physiological pH, glucuronides are readily excreted in urine. While excretion of the charged conjugate has traditionally been considered to be the primary role of the kidney in glucuronide disposition, accumulating evidence suggests that renal glucuronidation may significantly contribute to the metabolic clearance of drugs and other xenobiotics.[3–5] Several of the major drug metabolising glucuronosyltransferase (UGT) enzymes are expressed abundantly in human kidney, and UGT1A9 and UGT2B7 protein expression has been reported in the proximal and distal convoluted tubules, loops of Henle, macula densa, and collecting ducts.[6,7] Consistent with these observations, kidney microsomes have been shown to glucuronidate almokalant, carbamazepine, codeine, furosemide, gemfibrozil, morphine, mycophenolic acid, naloxone, paracetamol, propofol, valproic acid, and the nonsteroidal anti-inflammatory drugs flufenamic acid, flurbiprofen, ibuprofen, ketoprofen, mefenamic acid, naproxen and phenylbutazone.[4,7–16] Specific

Correspondence: Allan M. Evans, Division of Health Sciences, University of South Australia, City East, Adelaide, SA 5000, Australia.
E-mail: allan.evans@unisa.edu.au

activities for the glucuronidation of several of these drugs by renal microsomes were comparable with, or exceeded, those of hepatic microsomes.[9,11,12,14,15] Furthermore, it has been proposed that the kidney accounts for approximately one-third of the total metabolic clearance of the extensively glucuronidated drug propofol in humans after intravenous administration, and renal glucuronidation has been suggested as the main extrahepatic route of morphine elimination.[17,18]

While the in-vitro data support an important role for renal glucuronidation in drug elimination, few studies have investigated xenobiotic glucuronidation by the intact kidney. Moreover, differences in the renal kinetics of locally synthesised and circulating glucuronide (typically formed in the liver) has received little attention, despite the dual roles of the kidney in glucuronide formation and excretion.[19] The fate of generated and preformed glucuronides in the body may differ substantially. For example, the area under the plasma concentration curve, and hence systemic exposure, of generated metabolite is affected by the disposition of parent drug, but that of preformed metabolite is not. In addition, the distribution of preformed glucuronide will differ from that of renally formed metabolite due to differences in polarity of the conjugate and aglycone, UGT enzyme localization, diffusional barriers, transporters, and the contribution of glomerular filtration to preformed but not intra-renally synthesised glucuronide. Thus, administration of a preformed conjugate may not accurately reflect metabolite mediated toxicity, for example the proposed role of fenemate glucuronides in renal papillary necrosis.[10] Studies comparing the disposition of preformed and intrarenally synthesised glucuronides are therefore required to fully understand renal xenobiotic glucuronidation and its pharmacological and toxicological significance.

The utility of the isolated perfused kidney (IPK) with intact filtration capacity (filtering IPK) has been demonstrated in several studies from this and other laboratories.[20–23] Additionally, the nonfiltering IPK can provide important insights into renal drug and metabolite disposition. The nonfiltering IPK utilises a perfusate that creates high oncotic pressure to counterbalance filtration forces. Ligation of the ureter further ensures the absence of urinary excretion in the nonfiltering IPK. The nonfiltering IPK offers particular advantages for the investigation of drug transport via the basolateral membrane and demonstration of intra-cellular metabolism of drugs since there is no filtration, reabsorption and urinary excretion in the nonfiltering IPK.[24–26]

4-Methylumbelliferone (4MU) is a nonspecific substrate for most human UGT enzymes and has been employed to investigate the overall glucuronidation activity of kidney and liver microsomes.[27–29] In-vivo studies in the rat similarly indicated extensive glucuronidation of 4MU.[30,31] As in humans, however, the potential contribution of the kidney to 4MU glucuronide (4MUG) formation has been inferred from microsomal activity, and both rat liver and kidney appear to contribute to 4MUG formation.[32] However, this has not been confirmed in an intact kidney model.

Therefore, this study was designed to investigate the renal disposition of 4MU and 4MUG in the filtering IPK and nonfiltering IPK to characterise the contribution of excretion and metabolic clearance to total clearance in an intact kidney model, and to demonstrate differences in the renal handling of a preformed and renally synthesised glucuronide. The novel use of the nonfiltering IPK allowed us to demonstrate that luminal access of substrate had negligible impact on its overall renal metabolism. This suggested that renal impairment could lead to a lesser impact on renal drug metabolism than on excretory clearance.

Materials and Methods

Materials

4-Methylumbelliferone sodium salt (4MU), 4-methylumbelliferyl β-D-glucuronide (4MUG) hydrate, umbelliferone, [^{3}H]inulin, L-cysteine, glycine, L-glutamic acid and mannitol were purchased from Sigma-Aldrich (Sydney, Australia). Pluronic F108 (MW 14 600) was kindly donated by BASF Corporation (Florham Park, NJ, USA). D-Glucose and hydrochloric acid were purchased from AJAX Chemicals (Auburn, NSW, Australia). Sucrose and Tris HCl were supplied by BDH Chemicals (Poole, UK). Cellulose nitrate membrane filters were purchased from Sartorius (Gottingen, Germany), aqueous counting scintillant (ACS) from Amersham (Arlington Heights, IL, USA), Centricon Centrifugal Filter Devices (YM-10; molecular weight cut-off 10 000) from Millipore Corporation (Bedford, MA, USA), and Multiple Reagent Strips for Urinalysis from Bayer Diagnostics Business Group (Pymble, NSW, Australia). Water was purified using the Milli-RQ system (Bedford, MA, USA).

The rat isolated perfused kidney preparation

The study was approved by the Animal Ethics Committee of the Institute of Medical and Veterinary Science (IMVS; Adelaide, Australia). Male Sprague–Dawley rats (429–570 g), purchased from the IMVS, were maintained at 22–24°C on a 12-h light/dark cycle with free access to food and water. The filtering IPK preparation employed here was based on previously published methods.[33,34] The perfusion medium consisted of Krebs–Henseleit buffer (pH 7.4) containing Pluronic F108 (2.5 g/l), D-glucose (5 mmol/l), L-cysteine (0.5 mmol/l), glycine (2.3 mmol/l) and L-glutamic acid (0.5 mmol/l). The perfusion medium was filtered through a 0.45 μm filter before use and was equilibrated with a mixture of 95% O_2 and 5% CO_2 for 15 min before and throughout the perfusion.

Rats ($n = 10$) were anaesthetised by an intraperitoneal injection of sodium pentobarbitone (60 mg/kg). A midline laparotomy incision was made from the pelvis to the sternum and the right ureter was ligated immediately at the distal end. A solution of mannitol (150 mg) and heparin (100 U) in 1 ml normal saline was injected into the penile vein. The right ureter was then cannulated by the introduction of 200-mm tube (o.d. 0.61 mm, i.d. 0.28 mm). The area around the anastomosis of the superior mesenteric artery, renal artery and aorta was cleared of connective tissue and loose ligatures placed around the superior mesenteric artery and renal artery. After removing the capsule of the kidney, a right-angled glass cannula was inserted into the superior mesenteric artery via a small incision, and passed proximally along the superior mesenteric artery, across the aorta and into the right renal artery where it was tied in place. Perfusate flow was commenced immediately. The cannulated kidney was then excised

from the body of the rat and suspended within a thermostatically controlled cabinet at 37–38°C. The recirculating perfusion medium (160 ml) was pumped at 18–34 ml/min, which maintained perfusion pressure at 100 ± 20 mmHg. The functional viability of each filtering kidney was assessed by the glomerular filtration rate (GFR), which was determined as the renal clearance of [^{3}H]inulin and the percentage tubular reabsorption of water. The function of each filtering kidney was considered acceptable if the average value of GFR was greater than 0.25 ml/min and average percentage tubular reabsorption of water was greater than 50% during the 120-min perfusion period. For the nonfiltering IPK, the total renal clearances of 4MU and the formation rates of 4MUG were used as indicators of kidney viability when compared with the viable filtering IPK.

In addition, five nonfiltering IPKs were studied using a higher concentration of Pluronic F108 (5 g/l) in the perfusate and by ligating the proximal end of the right ureter.[24] All other procedures for the nonfiltering IPK were identical to those described for the filtering IPK.

Sample collection

A total of 15 perfusions were performed which included five filtering IPKs with 4MU, five filtering IPKs with preformed 4MUG, and five nonfiltering IPKs with 4MU. An equilibration time of 5 min was allowed after placing the perfused kidney in the thermostatic cabinet. [^{3}H]Inulin (130 kBq) was added to the perfusate reservoir and the perfusate volume in the recirculating system was adjusted to 160 ml by the addition or removal of fresh perfusate. For each perfusion, 0.16 ml 4MU (5.05 mM) or 0.8 ml 4MUG (2.84 mM) was added into the reservoir as a bolus to achieve initial ($t = 0$) concentrations of 50.5 and 14.2 μM, respectively.

Urine samples (for the filtering IPK) were collected over 10-min intervals from 10 to 120 min and perfusate samples (1.2 ml) collected from the reservoir at the midpoint of each interval. Urine volume was measured gravimetrically in preweighed vials and urine flow rate (UFR) was calculated accordingly. Concentrations of 4MU and 4MUG present in the perfusate, urine and kidneys were measured by HPLC. Kidneys were minced with scissors, homogenised in ice-cold buffered sucrose (0.25 M sucrose/20 mM Tris-HCl, pH 7.5; 5 ml/g tissue) with a MICCRA D-1 Homogeniser (ART, Mulheim, Germany) at 18 000 rev/min for 1 min. A sample (100 μl) of either perfusate, urine or kidney homogenate was mixed with 480 μl acetonitrile and 20 μl of a solution containing the internal standard (umbelliferone; 20 μg/ml). After centrifugation at 2000*g* for 10 min, 10 μl of the supernatant fraction was injected into the HPLC.

Binding in perfusate

Samples containing both 4MU (5.05 or 50.5 μM) and 4MUG (2.84 or 28.4 μM) in perfusate (containing either 2.5% or 5% F108) were prepared and allowed to equilibrate at 37°C for 30 min for determination of the unbound fractions (f_u) of 4MU and 4MUG in perfusate. Quadruplicate samples (1.8 ml) were dispensed into the chamber above the membrane of separate Centricon tubes and centrifuged at ambient temperature at 2000*g* for 60 min to obtain approximately 1 ml ultra-filtrate. The concentrations of 4MU and 4MUG in perfusate and ultrafiltrate were determined by HPLC. The f_u values for 4MU and 4MUG were calculated as the ratio of their respective concentration in ultrafiltrate to that in perfusate.

Analytical methods

A Shimadzu HPLC system (Shimadzu, Kyoto, Japan) consisted of a LC-10 ATvp pump, an autosampler, a RF-10AXL spectroflurometric detector set at excitation and emission wavelengths of 325 and 380 nm, respectively, and an alphaBond C_{18} column (10 μm, 300 × 3.9 mm; Alltech, Deerfield, Illinois, USA). Mobile phase, acetonitrile/20 mM NaH_2PO_4/1 M tetrabutylammonium bromide (20/80/4, v/v/v), was delivered at a flow rate of 1 ml/min at ambient temperature. Under these conditions, retention times for 4MUG, umbelliferone and 4MU were 5.7, 8.9 and 12.9 min, respectively. The lower limits of quantification for 4MU and 4MUG were 2.52 and 1.42 μM, respectively, which were determined as the lowest calibrator with intra- and inter-day precision (relative standard deviation) within 20% and accuracy within ±20% of the theoretical value.[35] The concentrations of 4MU and 4MUG in tissue were calculated as μmol/(kg tissue).

Calibration curves of peak area ratios (analyte : internal standard) against concentrations of analyte were linear ($r^2 > 0.999$) for 4MU (2.52–80.7 μM) and 4MUG (1.42–181.7 μM). The accuracy and precision for measured concentrations of quality controls spanning the calibration curve ranges were within ±20%. [^{3}H]Inulin in perfusate and urine was quantified by liquid scintillation spectrometry (Packard Model 2200CA, Canberra, Australia) following mixing of either 100 μl perfusate or urine with 3 ml aqueous counting scintillant.

Pharmacokinetic and statistical analyses

Noncompartmental methods in WinNonlin (Version 5.2, Pharsight Corp., Sunnyvale, California, USA) were used to calculate terminal half-life ($t_{1/2}$), area under the curve (AUC) from zero to 115 min (AUC_{last}) and from zero to infinite time (AUC_{inf}), and terminal volume of distribution (Vd_z) from concentrations of added substrate in the perfusion reservoir as a function of time. Total renal clearance ($CL_{R,total}$) was determined as dose divided by AUC_{inf}. The renal excretory clearance ($CL_{R,\ urine}$) of 4MU or 4MUG in each 10-min urine collection interval was determined as the rate of excretion into urine divided by the perfusate concentration of 4MU or 4MUG at the mid-point of the urine collection interval, according to equation 1:

$$CL_{R,urine} = \frac{[\text{Analyte}]_{\text{urine}} \times \text{UFR}}{[\text{Analyte}]_{\text{perfusate}}} \quad (1)$$

where $[\text{Analyte}]_{\text{urine}}$ and $[\text{Analyte}]_{\text{perfusate}}$ are the concentrations of analyte in urine and perfusate, respectively, and UFR is the urine flow rate. The renal excretory clearance of [^{3}H]inulin (GFR), was determined in an analogous manner. When 4MU was added to the perfusion reservoir, the urine and perfusate concentrations of 4MUG were used to determine renal excretory clearance of 4MUG, derived from both intra-renally generated and circulating 4MUG.

The clearance ratio (CR) for each analyte was calculated for each 10-min urine collection interval using equation 2:

$$CR = \frac{CL_{R,urine}}{f_u \times \mathrm{GFR}} \quad (2)$$

where fu is the unbound fraction in perfusate.

The metabolic clearance of 4MU was estimated by difference:

$$CL_{R,met} = CL_{R,total} - CL_{R,urine}$$

For the filtering IPK experiments, the percentage tubular reabsorption (%TR) of water was calculated using equation 3:

$$\%\mathrm{TR} = (1 - \mathrm{UFR}/\mathrm{GFR}) \times 100 \quad (3)$$

Concentrations of 4MU and 4MUG in the perfusate and urine samples collected in the last interval (110–120 min) were used for calculation of the respective concentration ratios between tissue and perfusate or urine.

Group data are presented as mean ± standard deviation (SD). Parameters across the two different groups or two different measures were compared using Student's *t*-test. Where indicated, a one-sample *t*-test was used to determine if $CL_R/(f_u \cdot \mathrm{GFR})$ was different from unity.

Results

The concentration of 4MU in perfusate declined with a terminal half-life of approximately 120 min following administration of a bolus dose (8.07 μmol) to the filtering IPK and nonfiltering IPK. There was a corresponding increase in the concentrations of 4MUG, which reached 15.8 ± 2.1 and 17.9 ± 5.0 μM at 115 min in the filtering IPK and nonfiltering IPK, respectively (Figure 1). 4MU bound to F108 in the filtering IPK and nonfiltering IPK perfusate ($f_u = 0.79$ and 0.67 for 2.5 and 5% F108, respectively), but 4MUG did not bind ($f_u = 1$). All pharmacokinetic parameters, total recovery (sum of 4MU and 4MUG in perfusate, urine and kidney as a proportion of the 4MU dose), and the formation clearance of 4MUG were close in value ($P \geq 0.75$) between the filtering IPK and nonfiltering IPK (Table 1).

The cumulative amount of 4MU excreted in urine during the 120-min perfusion was approximately 4% of the bolus dose and the renal excretory clearance of unchanged 4MU ($CL_{R,urine}$) accounted for 8% of the total renal clearance of 4MU from perfusate ($CL_{R,total}$) in the filtering IPK (Table 1). Thus, metabolic clearance of 4MU accounted for 92% of the total renal clearance of 4MU. Meanwhile, the unchanged 4MU retained in the kidney tissue at the end of perfusion accounted for approximately 2% of the bolus dose. During the initial 10-min collection interval, the efflux of generated 4MUG into perfusate (8.77 ± 4.55 mol/min) was 4.5-fold higher ($P < 0.05$) than that of urine (1.94 ± 1.30 nmol/min). The cumulative amount of 4MUG excreted in urine represented only 14% of the total amount of 4MUG formed, while the amount of 4MUG retained in the kidney tissue at the end of perfusion accounted for approximately 5% of the total 4MUG formed during the 120-min perfusion period.

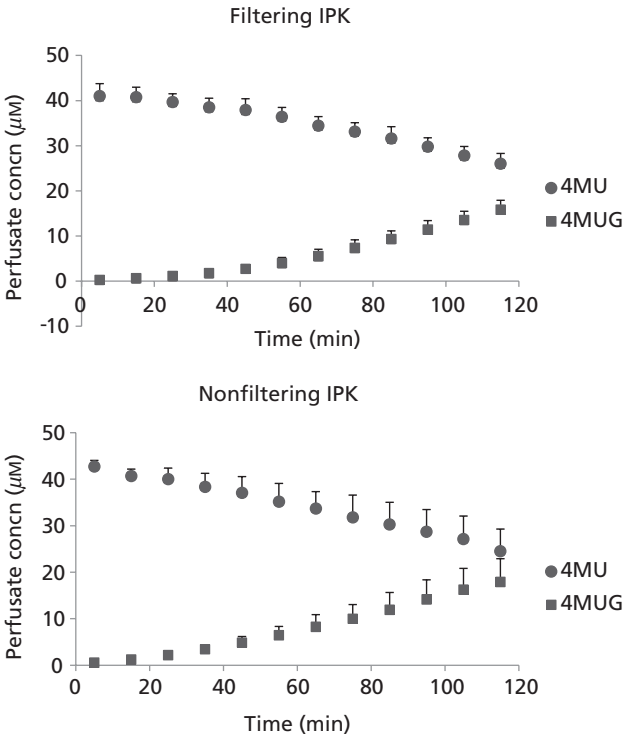

Figure 1 Perfusate 4-methylumbelliferone and 4-methylumbelliferyl glucuronide concentrations after bolus dosing (8.07 μmol) of 4-methylumbelliferone into the recirculation reservoir of the rat filtering or nonfiltering isolated perfused kidney. Data expressed as mean ± SD. $n = 5$ for each group. IPK, isolated perfused kidney; 4MU, 4-methylumbelliferone; 4MUG, 4-methylumbelliferyl glucuronide.

The time course of preformed 4MUG concentration in perfusate of the filtering IPK is presented in Figure 2 and relevant pharmacokinetic parameters are shown in Table 2. After bolus dosing (2.27 μmol) of preformed 4MUG in the perfusion reservoir of the filtering IPK, the perfusate concentration declined with the terminal half-life of approximately 260 min. The renal excretory clearance of preformed 4MUG accounted for 96% of total renal clearance. There was no detectable 4MU in the perfusate and urine samples collected from the filtering IPK with preformed 4MUG. Approximately 35% of the administered 4MUG was excreted into urine during the 120-min perfusion in the filtering IPK, while 3% of the 4MUG dose was retained in the kidney tissue at the end of perfusion.

The overall renal excretory clearance ($CL_{R,urine}$ 1.304 ± 0.356 ml/min) and renal clearance ratio ($CL_{R,urine}$/GFR, 3.758 ± 0.542) of generated 4MUG were significantly ($P < 0.05$) higher than that for the preformed 4MUG, as shown in Tables 1 and 2. The renal clearance ratio ($CL_{R,urine}/(f_u \cdot \mathrm{GFR})$) of 4MU (0.417 ± 0.044), generated 4MUG (3.758 ± 0.542), and preformed 4MUG (2.301 ± 0.816) were significantly ($P < 0.05$) lower (for 4MU) or higher (for 4MUG) than unity, suggesting that 4MU underwent net renal reabsorption, while 4MUG (both generated and preformed) underwent net renal secretion.

As shown in Figure 3, the 4MU and 4MUG (generated or preformed) concentrations in kidney tissue were higher than

Table 1 Pharmacokinetic parameters of 4-methylumbelliferone (bolus dose of 8.07 μmol) and its metabolite (4-methylumbelliferyl glucuronide) formed in the filtering and and nonfiltering isolated perfused kidney

Pharmacokinetic parameter	Filtering IPK ($n = 5$)		Nonfiltering IPK ($n = 5$)		P (t-test)
	Mean	SD	Mean	SD	
Recovery (sum of 4MU and 4MUG in perfusate, urine and kidney/dose of 4MU)	88.5%	4.6%	86.5%	3.7%	0.47
Total 4MUG generated/dose of 4MU	31.9%	3.2%	33.0%	9.0%	0.81
Formation clearance of 4MUG (ml/min)	0.670	0.13	0.715	0.27	0.75
Terminal half-life ($t_{1/2}$) of 4MU in perfusate (min)	117	39.0	123	36.2	0.83
Area under perfusate 4MU concentration curve from time 0 to 115 min (min*μmol/l)	4042	242	3981	399	0.78
Terminal volume of distribution (Vd_z) of 4MU (ml)	157	25.2	166	14.1	0.50
Total renal clearance ($CL_{R,total}$) of 4MU (ml/min)	0.980	0.192	1.00	0.30	0.90
Renal excretory clearance ($CL_{R,urine}$) of unchanged 4MU in urine (ml/min)	0.077	0.009	N/A	N/A	N/A
$CL_{R,urine}$/GFR of unchanged 4MU	0.417	0.044	N/A	N/A	N/A
Unchanged 4MU excreted in urine/dose of 4MU	3.96%	0.43%	N/A	N/A	N/A
Unchanged 4MU retained in kidney at the end of perfusion/dose of 4MU	1.91%	1.08%	ND	ND	N/A
Renal excretory clearance ($CL_{R,urine}$) of generated 4MUG through urine (ml/min)	1.30	0.36	N/A	N/A	N/A
$CL_{R,urine}$/GFR of generated 4MUG	3.76	0.54	N/A	N/A	N/A
Formed 4MUG excreted in urine/total 4MUG generated	13.9%	3.26%	N/A	N/A	N/A
Formed 4MUG retained in kidney at the end of perfusion/total 4MUG generated	4.89%	1.47%	ND	ND	N/A

GFR, glomerular filtration rate; IPK, isolated perfused kidney; 4MU, 4-methylumbelliferone ;4MUG, 4-methylumbelliferyl glucuronide; ND, not determined; N/A, not applicable.

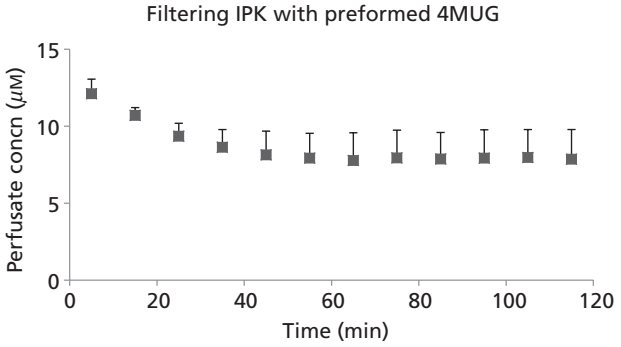

Figure 2 Perfusate 4-methylumbelliferyl glucuronide concentrations after bolus dosing (2.27 μmol) of preformed 4-methylumbelliferyl glucuronide into the recirculation reservoir of the rat filtering isolated perfused kidney. Data expressed as mean ± SD. $n = 5$. 4MUG, 4-methylumbelliferyl glucuronide.

Table 2 Pharmacokinetic parameters of preformed 4-methylumbelliferyl glucuronide (bolus dose of 2.27 μmol) in the filtering IPK $n = 5$

Pharmacokinetic parameter	Mean	SD
Recovery (4MUG in perfusate, urine andkidney/dose of 4MUG)	90.4%	7.1%
Terminal half-life ($t_{1/2}$) of 4MUG in perfusate (min)	263	166
Area under perfusate 4MUG concentration curve from time 0 to 115 min (AUC_{last}, min*μmol/l)	1005	156
Terminal volume of distribution (Vd_z) of 4MUG (ml)	221	12.1
Total renal clearance ($CL_{R,total}$) of 4MUG (ml/min)	0.78	0.41
Renal excretory clearance ($CL_{R,urine}$) of unchanged 4MUG through urine (ml/min)	0.75	0.39
$CL_{R,urine}$/GFR	2.30	0.82
4MUG excreted in urine/dose of 4MUG	34.6%	13.1%
4MUG retained in kidney at the end of perfusion/dose of 4MUG	2.84%	0.57%

4MUG, 4-methylumbelliferyl glucuronide.

the concentration in perfusate and urine. The tissue to urine 4MU concentration ratio was significantly higher than the concentration ratio between tissue and perfusate. Conversely, the 4MUG (generated or preformed) tissue to urine concentration ratio was considerably lower than the corresponding tissue to perfusate ratio (Figure 3). The tissue to perfusate and tissue to urine 4MU concentration ratios, and the 4MUG concentration ratio of tissue to perfusate were significantly greater than unity.

Discussion

This is the first study to employ both the IPK and nonfiltering IPK to characterise the relative contribution of metabolism and excretion to the renal elimination of a glucuronidated xenobiotic. The results provide direct evidence and important insights into the differential disposition of intra-renally generated and preformed glucuronides of lipophilic xenobiotics, and further demonstrate the ability of the kidney to glucuronidate xenobiotics. In the filtering kidney, the total clearance of 4MU was comprised mainly of metabolism via glucuronidation, with renal excretory clearance ($CL_{R,urine}$) of unchanged 4MU accounting for only 8% of total renal clearance. Thus, approximately 90% of the 4MU in perfusate was cleared by glucuronidation. These data were consistent with in-vitro studies suggesting that renal glucuronidation contributed significantly to the metabolic clearances of many compounds eliminated via this pathway.[4] Previous IPK and in-vivo studies support the role of intra-renal glucuronidation

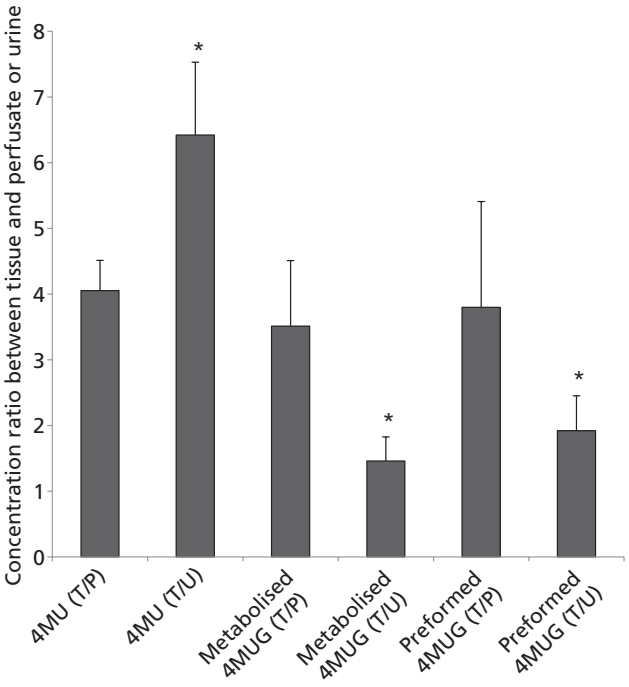

Figure 3 The concentration ratios of 4-methylumbelliferone, intrarenal generated 4-methylumbelliferyl glucuronide, or administered preformed 4-methylumbelliferyl glucuronide between tissue and perfusate or between tissue and urine in the filtering isolated perfused rat kidney at the end of perfusion. 4-Methylumbelliferone, 4MU; 4-methylumbelliferyl glucuronide, 4MUG; tissue and perfusate, T/P; tissue and urine, T/U. $^*P < 0.05$ compared with the corresponding ratio of T/P.

in the elimination of the xenobiotic phenols 1-naphthol and 4-nitrophenol in the rat.[36–39]

The glucuronidation of 4MU in renal tubule cells was consistent with the reported distribution of UGTs in kidney.[7] In the filtering IPK, 4MU can enter renal tubule cells through the basolateral membrane directly from perfusate and indirectly via the luminal membrane from the tubule lumen. By contrast, 4MU is only able to enter the cell through the basolateral membrane in the nonfiltering IPK. However, the total renal clearances of 4MU and the formation clearance of 4MUG in the filtering IPK and nonfiltering IPK were very similar (Table 1), indicating that the influx of 4MU from basolateral uptake far exceeded that entering via luminal reabsorption. The data suggested that 4MU did not rely to any significant extent on reabsorption from the tubule lumen to access the intracellular sites of glucuronidation, which would be expected if the total clearance of 4MU in the filtering kidney was substantially greater than that in the nonfiltering IPK. This suggested that kidney disorders which mainly impair glomerular filtration (e.g. acute glomerulonephritis) would alter renal metabolic clearance to a much lesser extent.

The clearance ratio (*CR*) is more informative than renal excretory clearance and extraction ratio for investigating the mechanisms involved in the renal excretory clearance of a substance, since the GFR in the filtering IPK varies between different preparations and can change with time within a single perfused kidney.[40] The *CR* value for 4MU (0.417 ± 0.044) was significantly less than unity, indicating that 4MU underwent net tubular reabsorption in the filtering IPK. On the other hand, the *CR* value for preformed 4MUG (2.301 ± 0.816) exceeded unity, indicating net tubular secretion of 4MUG. These data accord with our findings that the 4MU tissue to urine concentration ratio was higher than that of tissue to perfusate, while the 4MUG tissue to urine ratio was lower than tissue to perfusate. Since the 4MU tissue to urine and tissue to perfusate concentration ratios and the 4MUG tissue to perfusate concentration ratios were greater than unity (Figure 3), 4MU reabsorption via the luminal membrane and uptake of 4MU and 4MUG (preformed) through the basolateral membrane are expected to be active-transport mediated processes, although tissue binding/partitioning phenomena can also influence these ratios. Although the concentrations of 4MU and 4MUG in kidney tissues were higher than those in perfusate and urine, due to the small mass of kidney (<2.5 g) relative to the volume of perfusate (160 ml), the amount of 4MU and 4MUG retained in the kidney tissue at the end of perfusion was still relatively low, i.e. <3% of the bolus dose of 4MU or of preformed 4MUG for unchanged 4MU or 4MUG in the tissue, respectively, or <5% of the total amount of MUG formed in the filtering IPK for the generated 4MUG retained in the tissue (Tables 1 and 2).

The renal excretory clearance of intrarenal generated 4MUG was significantly higher than that of preformed 4MUG (Tables 1 and 2). This may have been due to the fact that preformed 4MUG was excreted primarily via glomerular filtration and tubular secretion, whereas intrarenal generated 4MUG could also efflux directly into tubule lumen after being generated *in situ*. It should be noted that the dose of preformed 4MUG was chosen to achieve its initial perfusate concentration (14.2 μM), which was comparable with the highest concentration (average 15.8 μM) of intra-renal generated 4MUG in perfusate after dosing 4MU to the filtering IPK in this study to minimise the impact of any potential nonlinearity.

Investigation of the fate of glucuronide conjugates generated in the tubule cells was important for understanding the role of the kidney as a detoxification organ. In this study, the efflux of 4MUG into perfusate was significantly higher than that into urine during the initial collection period of the 4MU administration experiment in filtering IPK mode. Moreover, only 14.5% of total 4MUG recovered in these experiments was excreted into urine during the 120-min perfusion. This may have been due to differences in the affinity and/or capacity of 4MUG transporters located on the basolateral and luminal membranes of the tubule cells. It has been reported that a variety of glucuronides are substrates for Mrp1, Mrp2 and Mrp3, which mediate efflux of glucuronide across the basolateral and luminal membranes.[41] Our results were consistent with a previous study in which the generated 1-naphthol glucuronide was preferentially excreted into perfusate (plasma) rather than into urine in the rat filtering IPK after administration of 1-naphthol.[37]

Conclusions

We have shown that 4MU was extensively metabolized by glucuronidation in the filtering and nonfiltering IPK, and that the renal clearance of 4MU was far greater than the renal

clearance of the metabolite. This indicated that glucuronidation was the major elimination pathway for 4MU in the kidney, and confirmed accumulating in-vitro data for substantial renal metabolic clearance of drugs and other xenobiotics via glucuronidation. The influx of 4MU from basolateral uptake far exceeded that entering via luminal reabsorption and 4MU did not rely to any significant extent on reabsorption from the renal tubule to access the intracellular sites of glucuronidation. 4MU underwent net tubular reabsorption whereas 4MUG underwent net tubular secretion in the filtering IPK. The study has reinforced the potential role of the kidney in the clearance of drugs and other xenobiotics and signals the lack of impact of impaired glomerular filtration on renal drug metabolism.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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