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CONJUGATION-DECONJUGATION CYCLING OF DIFLUNISAL VIA ß-GLUCURONIDASE CATALYZED HYDROLYSIS OF ITS ACYL GLUCURONIDE IN THE RAT

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Summary

The role of ß-glucuronidase catalyzed hydrolysis of glucuronides on the in vivo disposition kinetics of xenobiotics was studied in the rat. The metabolic disposition kinetics of diflunisal, a compound undergoing transformation to an acyl and phenyl glucuronide, were studied in rats under control conditions and following administration of D-glucaro-1,4-lactone, a potent and specific ß-glucuronidase inhibitor. D-glucaro-1,4-lactone treatment resulted in a significant decrease in ß-glucuronidase activity in plasma, urine, and hepatic microsomes. Total (i.e. urinary and biliary) recovery of the acyl glucuronide following i.v. injection of diflunisal (10 mg/kg) was significantly higher in D-glucaro-1,4-lactone treated rats (41 ± 3%, n=6) compared to control rats (29 ± 2%, n=6) whereas for diflunisal phenyl glucuronide this total recovery was very similar in both groups of rats (16.0 ± 1.0% vs. 18.0 ± 0.2%, n=6, respectively). The partial clearance of diflunisal associated with the formation of the acyl glucuronide was significantly higher in D-glucaro-1,4-lactone treated rats (0.413 ± 0.024 ml/min/kg) compared to control animals (0.269 ± 0.042 ml/min/kg). The partial clearance related to the formation of the phenyl glucuronide, on the contrary, was not significantly affected by D-glucaro-1,4-lactone treatment. These results shows that the in vivo glucuronidation of diflunisal to the acyl glucuronide, unlike diflunisal glucuronidation to the phenyl glucuronide, is subject to a futile conjugation-deconjugation cycle. Such futile cycling may have significant therapeutic and toxic implications.

Key Words: **glucuronidation-deglucuronidation, β-glucuronidase, diflunisal**

UDP-Glucuronosyltransferases (UGT; EC 2.4.1.17) are a family of closely related enzymes mainly located in the endoplasmic reticulum of the liver and various extrahepatic tissues such as the small intestine, kidney, lung, etc (1,2). They catalyse the conversion of numerous lipophilic endo- and xenobiotics into water-soluble products that are readily excreted in urine and/or bile. Most glucuronide conjugates are good substrates for ß-glucuronidase (ßG; EC 3.2.1.31), a hydrolase present in most mammalian tissues (3). ßG is mainly a lysosomal enzyme but is also located in the endoplasmic reticulum (4). The close intracellular proximity of the UGT's, responsible for the synthesis of the glucuronides, and ßG, mediating the hydrolysis of the same glucuronides to liberate their aglycone, creates the possibility for a futile conjugation-deconjugation cycle (5). Although such futile conjugation-deconjugation cycling has been demonstrated by in vitro techniques for a

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number of compounds such as p-nitrophenol (6), 4-methylumbelliferone (7,8), 3-benzo[a]pyrene (9) and diflunisal (10), the possible contribution of reversible metabolism via glucuronide hydrolysis to the in vivo elimination of these compounds remains largely unexplored.

Diflunisal (DF), a difluorophenyl derivative of salicylic acid, is mainly eliminated by formation of two glucuronides and a sulfate conjugate (11,12) (Fig. 1). We recently showed that diflunisal acyl glucuronide (DAG), unlike the phenyl glucuronide (DPG), was very rapidly hydrolyzed by rat liver microsomal βG (10). The purpose of this study was to determine whether the conjugation-deconjugation cycling of diflunisal in rat liver microsomes is also occurring in vivo and, if so, is sufficiently important to significantly affect the overall rate of metabolic elimination of this salicylate derivative. Therefore, pharmacokinetic/metabolic studies were carried out in control rats and rats being treated with D-glucaro-1,4-lactone (GL), a potent and specific βG inhibitor (13). Since GL is excreted in the bile (14) and may thus affect the pharmacokinetics of diflunisal which undergoes enterohepatic cycling in the rat via biliary excretion of its glucuronides (12,15), the experiments were carried out in bile-exteriorized animals

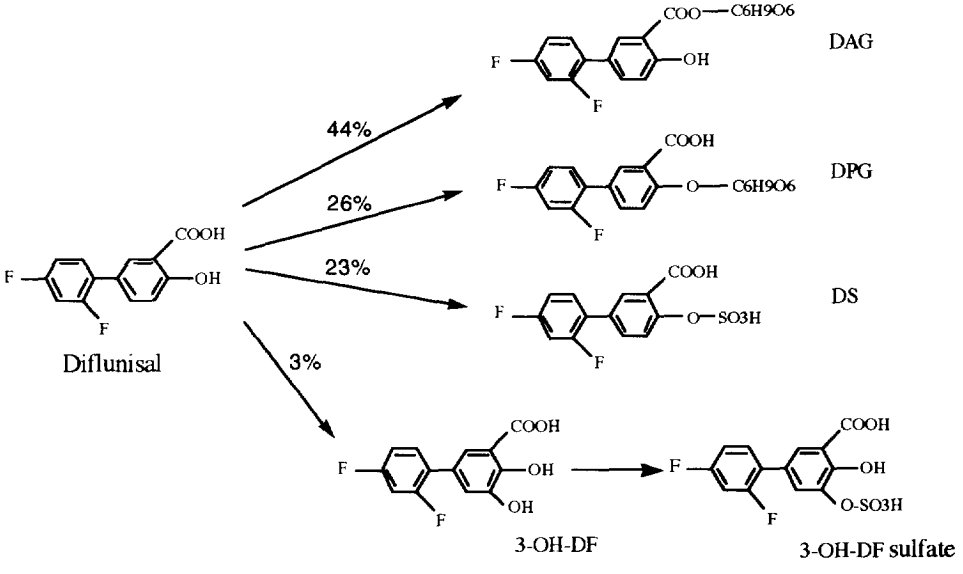

Fig. 1

Metabolic fate of diflunisal in the rat; the numbers indicate the approximate percentage of the recovered dose, after i.v. injection of 10 mg/kg, converted to a particular metabolite (20)

Materials and Methods

Chemicals.

Diflunisal and D-glucaro-1,4-lactone were purchased from Sigma Chemical Co. (St. Louis, MO). DAG, DPG and DS were isolated from human urine following oral ingestion of a 500 diflunisal dose as described before (16). Pure 3-hydroxy-diflunisal was kindly donated by Dr. R. G. Dickinson (The University of Queensland, Brisbane, Australia). HPLC grade methanol and acetonitrile were purchased from Labscan (Dublin, Ireland). All other chemicals used were of the highest purity available from commercial sources.

Animals and treatments.

Male Wistar rats, weighing between 270 and 290 grams, were supplied by the University Breeding Facilities. The animals were maintained at 21 ± 2 °C on a 12 hour light-dark cycle and had free access to food (A03, UAR, France) and water. Under pentobarbital anesthesia (55 mg/kg i.p.), Silclear^{R} catheters (0.020" x 0.037") were placed in the 2 external jugular veins, one for i.v. administration and the other for blood sampling. In addition a PE-50 cannula was inserted into the bile duct for bile collection. After surgery, rats were allowed to recover for 4 hours before the start of the pharmacokinetic experiment. Two groups of 6 rats were studied. The control group received an i.v. bolus dose of diflunisal (10 mg/kg). A second group of 6 animals received the same dose of diflunisal but in addition was treated with D-glucaro-1,4-lactone, a ß-glucuronidase inhibitor, dissolved in 0.065 M $NaHCO_3$ (pH 7.4) as follows: 11 bolus injections of 50 mg/kg at -1, -0.5, 0, 1, 2, 3, 4, 5, 6, 7 and 8 hours relative to the i.v. injection of diflunisal. The control rats received 11 bolus injections of 0.065 M $NaHCO_3$ (pH 7.4) using the same administration schedule. Blood samples (200 μl) were withdrawn in a heparinized syringe at the following times: 0 (pre-dose), 2.5, 5, 10, 15, 20 and 30 min and 1, 2, 3, 4, 5, 6 and 8 hours following diflunisal administration. Blood samples were immediately centrifuged and the plasma was frozen at - 20 °C until analysis. Bile was collected on ice during hourly intervals into preweighed vials containing 0.2 ml of 1 M acetic acid. Bile collection was stopped at 8 hours following diflunisal administration since preliminary results had shown that biliary excretion of diflunisal and its glucuronides was virtually complete at that time. Urine was also collected on ice into vials containing 1 ml 1 M acetate buffer pH 5 and during the following intervals: 0-4, 4-8, 8-24 hours. Bile and urine samples were immediately frozen at - 20 °C until analysis.

ß-Glucuronidase assay.

The ß-glucuronidase activity was measured in urine and bile samples of control and D-glucaro-1,4-lactone treated rats using 4-methylumbelliferone glucuronide as substrate (17). To measure the ß-glucuronidase activity in plasma and liver microsomes, additional experiments were carried out in 6 rats, i.e. 3 control and 3 D-glucaro-1,4-lactone treated rats. These animals did not receive diflunisal but were otherwise treated exactly the same way as described above (including catheter placement). They were killed by decapitation 1.5 hours after starting the $NaHCO_3$ (pH 7.4) or D-glucaro-1,4-lactone treatment. At that time a blood sample was taken and the liver was removed to prepare microsomes as described by Amar-Costesec (18). ß-Glucuronidase activity in plasma was measured by the method of Mead (17). Microsomal ß-glucuronidase activity, however, was measured by a different method with phenolphthalein glucuronide as substrate (10).

DAG and DPG stability in urine.

After adjusting the pH to 6.0 or 7.4 with the same volume of diluted HCl, urine samples of untreated rats were spiked with DAG or DPG to obtain 75 μg/ml and incubated at 37 °C in the absence or the presence of 4 mM D-glucaro-1,4-lactone. Control experiments were carried out in phosphate buffer pH 6.0 and 7.4. An aliquot of the urine or buffer solution was sampled at regular intervals and the diflunisal concentrations were determined by HPLC.

HPLC analysis.

Concentrations of DAG, DPG and DS in bile and urine were determined using the method of Dickinson and King (19). However, since the concentrations of DF in bile and urine were very low, fluorescence detection was chosen (excitation and emission wavelength: 258 and 428 nm, respectively) instead of uv detection. The concentrations of DF, DAG, DPG and DS in plasma were determined by a modification of the method of Dickinson and King using two different HPLC systems. For the determination of DF, DPG and DS, the HPLC system was composed of a pump (325-system, Kontron, Zurich, Switzerland), a Novapak C_{18} 4 μm cartridge inserted in a RCM-100 radial compression module (Waters Associates, Milford, MA, USA) and a fluorescence detector (SL-40, Perkin-Elmer, Beaconsfield, UK). The mobile phase was composed of methanol and phosphate buffer (0.01 M Na_2HPO_4 adjusted to pH 2.7 with orthophosphoric acid and containing Na_2SO_4.10 H_2O 4% wt/vol). Initially, the mobile phase was composed of buffer/methanol 62/38 and after 12 minutes was changed to 49/51 v/v. The mobile phase was pumped at a flow rate of 2.5 ml/min. The eluate was monitored fluorimetrically at 235 and 370 nm (excitation and emission wavelength, respectively). For the determination of DAG, the HPLC system was composed of a pump (420-system, Kontron), a Hypersil ODS 5 μm column 250 x 4.6 mm (Alltech Associates,

Deerfield, IL, USA) and a UV detector (433, Kontron). The mobile phase (55/45 v/v methanol/phosphate buffer 0.01 M Na_2HPO_4 adjusted to pH 2.7 with orthophosphoric acid and containing $Na_2SO_4.10\,H_2O$ 1% wt/vol) was pumped through the column at 1 ml/min and the eluate was monitored at 226 nm. The plasma samples were prepared by mixing 2 volumes of plasma with 3 volumes of the internal standards solution (clofibric acid 1 μg/ml and α-naphtol glucuronide 5 μg/ml dissolved in acetonitrile containing 4% acetic acid, for the UV and fluorimetric detection respectively). Aliquots of the supernatant were injected onto both HPLC systems described above. Concentrations of OH-diflunisal in urine were determined using the method described previously by Dickinson et al. (20) but using a fluorescence detector at the same wavelengths as used for determination of DF in bile and urine. The concentrations of OH-diflunisal and DS in bile were below the limit of quantification.

Data analysis.

The terminal plasma half-life of DF ($t_{1/2z}$) was determined from the slope (λz) of the terminal linear portion of the log diflunisal plasma concentration vs. time profile. Plasma clearance (CL) and apparent distribution volume (Vd) of diflunisal were calculated as dose/AUC and dose/AUC. λz, respectively, where AUC is the area under the diflunisal plasma concentration-time profile extrapolated to infinity. Partial clearances of DF corresponding to the formation of DF metabolites ($CL_{f,m}$) were calculated as the product of CL and $f_{m,r+b}$, the fraction of the i.v. dose of DF recovered in urine and bile as a particular metabolite m. Renal (Cl_r) and biliary clearance (Cl_b) of DF and its metabolite were calculated as the amount of DF or metabolite recovered in urine or bile divided by the AUC of DF or metabolite

Statistics.

All results are expressed as the mean ± S.E.M.. Mean values of pharmacokinetic parameters in the control and the D-glucaro-1,4-lactone treated rats were compared by unpaired t-test.

Results

Treatment with D-glucaro-1,4-lactone did not affect bile production (data not shown), but urine pH was significantly reduced following treatment with the ß-glucuronidase inhibitor (control: 7.40 ± 0.28, GL treatment: 6.00 ± 0.17, $P<0.05$).

ß-Glucuronidase activity was significantly decreased in liver microsomes, plasma and urine of D-glucaro-1,4-lactone treated rats (table 1). This decrease in ß-glucuronidase activity was particularly spectacular in urine where its activity in treated rats was almost completely inhibited by D-glucaro-1,4-lactone. In bile, on the contrary, ß-glucuronidase activity was much smaller than in urine and was not significantly reduced by D-glucaro-1,4-lactone treatment.

Table I

ß-Glucuronidase Activity

	Controls	GL-Treated
Microsomes (Fu/mg proteins)	86.1 ± 5.3	61.6 ± 3.1*
Plasma (u/ml)	1.60 ± 0.10	0.99 ± 0.17*
Urine (u/ml)	11.476 ± 3.130	0.002 ± 0.002*
Bile (u/ml)	0.02 ± 0.012	0.01 ± 0.007

Fu/mg protein= Fishman units/mg of microsomal proteins; u/ml =μg of 4-methylumbelliferone liberated per hour per ml of plasma, urine or bile, *$P<0.05$

Figure 2 shows the plasma concentration-time profiles for DF, DAG, DPG and DS in control and D-glucaro-1,4-lactone treated rats. The major pharmacokinetic parameters of DF were not significantly affected by glucaro-1,4-lactone treatment (table 2).

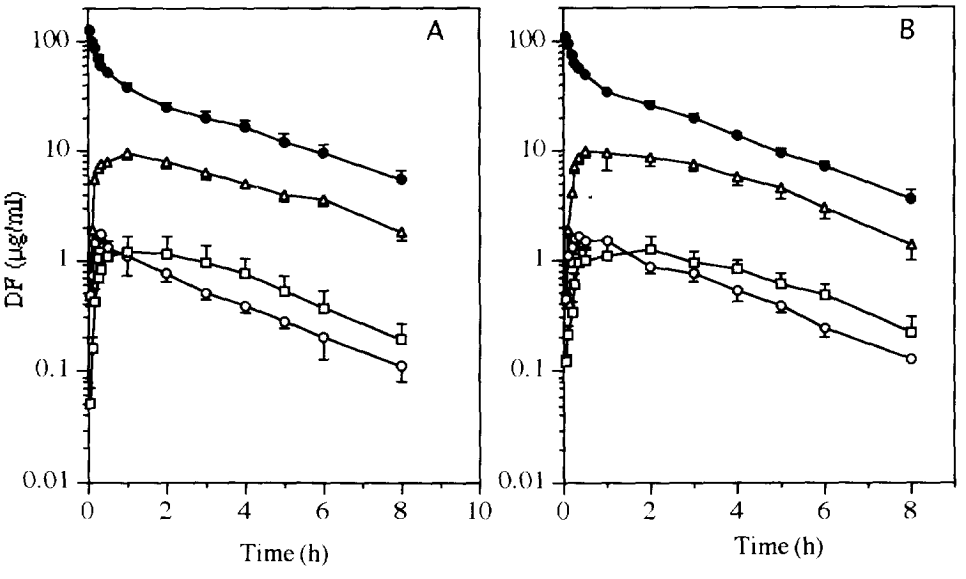

Fig. 2

Plasma concentration-time profiles for diflunisal (•) and its metabolites (DAG (○), DPG (□)DS (Δ) after i.v. administration of diflunisal 10 mg/kg to bile-exteriorized rats. A: control rats (n=6); B: GL-treated rats (n=6).
Concentrations of DAG, DPG and DS are expressed as diflunisal equivalents.

Table II

Effect of D-glucaro-1,4-lactone on the Plasma Pharmacokinetics of Diflunisal

	Controls	**GL-Treated**
CL (ml/min/Kg)	0.93 ± 0.13	1.02 ± 0.07
Vd (ml/kg)	222.4 ± 31.3	186.1 ± 14.5
λ_z (h^{-1})	0.282 ± 0.066	0.336 ± 0.030

Biliary and urinary recoveries of DF and its metabolites are summarized in table 3. In control rats, both diflunisal glucuronides were excreted in urine and bile. For DAG the fraction of the DF dose recovered in bile (f_b: 0.18 ± 0.02) was larger than the fraction recovered in urine (0.11 ± 0.03), whereas for DPG both fractions were the same, i.e. 0.08 ± 0.01. D-Glucaro-1,4-lactone treatment significantly increased f_b for DAG (+ 62%) without affecting f_r. Biliary and urinary recoveries of DPG were not affected by D-glucaro-1,4-lactone treatment. DS and 3-OH-DF were only excreted via urine (f_r: 0.33 ± 0.02 for DS and 0.021 ± 0.002 for 3-OH-DF) and their urinary recoveries were not influenced by D-glucaro-1,4-lactone treatment. Finally, excretion of unchanged DF was small in

urine (0.043 ± 0.008) and bile (0.004 ± 0.001) of control rats. D-Glucaro-1,4-lactone treatment significantly decreased f_r (0.006 ± 0.001) whereas f_b was not significantly affected (0.006 ± 0.001). The overall recovery (i.e. urinary plus biliary) of DF and its metabolites was 0.85 ± 0.03 and was significantly higher (0.95 ± 0.03, P< 0.05) in D-glucaro-1,4-lactone treated rats.

Table III

Urinary and Biliary Recoveries of Diflunisal and its Metabolites

	f r	f b	f r+b
DF			
Controls	0.043 ± 0.008	0.004 ± 0.0006	0.047 ± 0.008
GL-Treated	0.006 ± 0.001 **	0.006 ± 0.0006	0.011 ± 0.007
DAG			
Controls	0.11 ± 0.03	0.18 ± 0.02	0.29 ± 0.02
GL-Treated	0.10 ± 0.01	0.29 ± 0.04 *	0.41 ± 0.03**
DPG			
Controls	0.08 ± 0.01	0.08 ± 0.01	0.16 ± 0.010
GL-Treated	0.08 ± 0.02	0.10 ± 0.01	0.18 ± 0.002*#
DS			
Controls	0.33 ± 0.02	ND	0.33 ± 0.02
GL-Treated	0.33 ± 0.01	ND	0.33 ± 0.01
OH-DF			
Controls	0.021 ± 0.002	ND	0.021 ± 0.002
GL-Treated	0.018 ± 0.002	ND	0.018 ± 0.002
DF + metabolites			
Controls	0.580 ± 0.034	0.264 ± 0.032	0.844 ± 0.03
GL-Treated	0.533 ± 0.040	0.418 ± 0.036**	0.949 ± 0.029*

f_b, f_r, f_{r+b} is the fraction of dose of diflunisal excreted as diflunisal itself or as its metabolites in bile, urine or bile and urine together, respectively.
*P<0.05, **P<0.005, # Two-tailed unpaired t-test for unequal variance (Fisher test)
ND: not detectable

The partial clearances for the formation of DAG, DPG, DS and the 3-OH-DF and the biliary and renal clearances of DF are summarized in table 4. Only the partial clearance related to the formation of DAG was significantly affected by treatment with the ß-glucuronidase inhibitor: 0.413 ± 0.024 ml/min/kg in treated rats versus 0.269 ± 0.042 ml/min/kg (P< 0.05). The renal clearance of DF was significantly reduced by glucaro-1,4-lactone treatment (P< 0.05). D-glucaro-1,4-lactone treatment did not significantly affect the biliary and/or the renal clearances (in ml/min/kg) of DPG (Cl_r: 2.71 ± 0.49 vs 2.74 ± 0.96; Cl_b: 4.01 ± 1.29 vs 2.71 ± 0.87 in control and GL-treated rats, respectively), DAG (Cl_r:3.18 ± 0.78 vs 3.08 ± 0.55; Cl_b: 6.41 ± 1.03 vs 9.74 + 2.37) and DS (Cl_r: 1.16 + 0.11 vs 1.29 ± 0.17).

Stability experiments were carried out with DAG and DPG in urine of untreated rats adjusted to pH 6.0 and 7.4 and in phosphate buffer at different pH (6.0 and 7.4) to investigate whether the significant decrease in urinary excretion of unchanged DF in D-glucaro-1,4-lactone treated rats was due to inhibition of urinary ß-glucuronidase activity or due to a pH effect. The results show that hydrolysis of DAG in buffer is rather slow but occurs at a faster rate at pH 7.4 compared to pH 6.0. In contrast, hydrolysis of DAG in urine is much faster compared to buffer and is favored at pH 6.0. In some rats hydrolysis of DAG is so fast that more than 50% of DAG added is hydrolyzed after 30 minutes incubation at 37 °C. Addition of 4 mM glucaro-1,4-lactone almost completely inhibited DAG hydrolysis in urine (table 5). For DPG, no measurable hydrolysis occurred at 37 °C in buffer pH 6.0 and 7.4 during a 24-hour period. Hydrolysis of DPG in urine was also very small: 4.0% hydrolyzed during 24 hours at pH 6.0 and 0.5 % at pH 7.4 (n=2). D-glucaro-1,4-lactone completely inhibited this hydrolysis.

Table IV

Partial Clearances for the Formation of DAG, DPG, DS, 3-OH-DF and Biliary and Renal Clearances of DF

	Controls	GL-Treated
$CL_{f,\ DPG}$ (ml/min/kg)	0.141 ± 0.015	0.174 ± 0.012
$CL_{f,\ DAG}$ (ml/min/kg)	0.269 ± 0.042	0.413 ± 0.024*
$CL_{f,\ DS}$ (ml/min/kg)	0.307 ± 0.040	0.336 ± 0.027
$CL_{f,\ OH\text{-}DF}$ (ml/min/kg)	0.020 ± 0.004	0.021 ± 0.002
$CL_{r,\ DF}$ (ml/min/kg)	0.042 ± 0.012	0.0057 ± 0.001*
$CL_{b,\ DF}$ (ml/min/kg)	0.0036 ± 0.0005	0.0056 ± 0.0006*

*$P<0.05$

Table V

Effect of D-glucaro-1,4-lactone on Hydrolysis of DAG after 30 Minutes Incubation at 37 °C in Urine and Buffer at pH 7.4 and 6.0

Sample	pH 7.4		pH 6.0	
	Control	4 mM GL	Control	4 mM GL
urine 1	68.4	-	98.8	-
urine 2	4.6	-	54.0	-
urine 3	7.2	-	15.2	-
urine 4	6.8	5.2	14.2	1.4
urine 5	54.9	5.7	74.8	2.4
urine 6	13.8	4.1	66.3	1.0
buffer (n=4)	4.0 ± 0.8		2.1 ± 0.3	

Results are expressed as percent DAG hydrolyzed; -: not determined.

Discussion

Inasmuch as glucuronidation plays an important role in the detoxification of many endo- and xenobiotics, including carcinogens, it is necessary to examine the possible contribution of deglucuronidation to the overall elimination of these compounds. Although a glucuronidation-deglucuronidation cycle has been shown in vitro for a few compounds (6-10), and the importance of such cycling in controlling the elimination and thus toxicity and/or therapeutic efficacy of endo- and xenobiotics has been postulated (5,21,22), little experimental evidence exists regarding the in vivo deconjugation of glucuronides with the exception of bilirubin glucuronide. As early as 1968, Acocella et al. (23) and Okolicsanyi et al. (24) demonstrated that Gunn rats, which are congenitally deficient in the UGT isozymes responsible for bilirubin glucuronidation, were able to deconjugate bilirubin glucuronide following its i.v. administration. No evidence was provided, however, whether hepatic or extrahepatic ß-glucuronidase was involved. More recently, Whiting et al. (25) utilized two unique congenic strains of mice, one with almost complete hepatic ßG deficiency and

the second lacking hepatic microsomal ßG activity, to characterize the role of hepatic ßG in the metabolism and disposition of bilirubin-IXα. They found a twofold increase in the biliary excretion of bilirubin-IXα monoglucuronide in both types of the mutant mice compared to the controls. Their results demonstrated that microsomal ßG, unlike lysosomal ßG, modulates the net rate of bilirubin-IXα glucuronidation and glucuronide excretion in bile.

We used inhibition of ßG activity with GL to investigate the possible role of a glucuronidation-deglucuronidation futile cycle in the metabolic disposition of diflunisal in the rat. The partial clearance of diflunisal related to the formation of DAG was increased by 65% in GL-treated rats demonstrating the modulating role of ß-glucuronidase catalyzed hydrolysis in the overall formation of this acyl glucuronide. Interestingly, the partial clearance of diflunisal due to the formation of phenyl glucuronide of diflunisal was not significantly affected by GL treatment. This is consistent with in vitro results on rat liver microsomes (10). In contrast to DAG which was very rapidly hydrolyzed by rat liver microsomes ($t_{1/2}$: ca. 12 min), DPG hydrolysis was extremely slow ($t_{1/2}$: ca. 35 hours). Consequently, glucuronidation-deglucuronidation futile cycling is only of practical importance for those glucuronides exhibiting a hydrolysis rate comparable to its rate of synthesis. If the rate of hydrolysis of the glucuronide conjugate is much slower than its formation rate, competing pathways for glucuronide elimination, such as renal and biliary excretion, will efficiently remove the glucuronide from the circulation.

GL treatment significantly decreased the ßG activity in plasma, urine and liver microsomes. The decrease in ßG activity observed in bile was not statistically significant. The urinary ßG activity, on the contrary, was decreased by GL treatment to less than 1% of the control activity! Inhibition of ßG activity in urine by GL treatment leading to reduced hydrolysis of DAG in the bladder explains the significant decrease in urinary recovery of unchanged diflunisal, from 4.3% of the administered dose in control rats to 0.6% in GL-treated rats. Additional in vitro stability studies with DAG and DPG in urine were consistent with these results. Acyl glucuronide conjugates have been shown to be reactive metabolites capable of undergoing non-enzymatic hydrolysis, rearrangement (isomerization via acyl migration) and covalent binding with plasma and tissues proteins (26). Dickinson and King (27) showed that covalent binding of diflunisal to the bladder tissue of the rat was mediated by DAG, the reactive acyl glucuronide of diflunisal. Urinary pH and urinary ßG activity play a modulating role in the formation of covalent adducts of diflunisal with urinary bladder tissue. The same authors also demonstrated that diflunisal undergoes facile absorption from the urinary bladder into the blood of the rat (28). This may explain why the urinay recovery of unchanged diflunisal in the rat is small (less than 5% of the administered dose) despite relatively rapid ßG catalyzed hydrolysis of DAG in certain urine samples even at physiologic urine pH. In general, ßG catalyzed hydrolysis of glucuronide conjugates in the bladder may liberate potentially toxic aglycones thus promoting bladder toxicity.

In summary, we have clearly shown that a glucuronidation-deglucuronidation cycle occurs with diflunisal in vivo in the rat. In addition, it was demonstrated that it only involved the formation of the acyl glucuronide and not the formation of the phenyl glucuronide. This futile cycle did not significantly affect the pharmacokinetic behavior of diflunisal because this drug is eliminated by multiple parallel metabolic pathways. The existence of such a glucuronidation-deglucuronidation futile cycle may, however, be important for compounds mainly eliminated by formation of a ßG-sensitive glucuronide. In that case the ßG catalyzed hydrolysis of the glucuronide will significantly prolong the mean residence time of parent substance in the body which may have therapeutic or toxic implications.

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