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Gender Is an Important Determinant of the Disposition of the Loop Diuretic Torasemide

Ulrike Werner, PhD, Dierk Werner, MD, Svetlana Heinbüchner, MD, Bernhard Graf, MD, Hüseyin Ince, MD, Stefan Kische, MD, Petra Thürmann, MD, Jörg König, PhD, Martin F. Fromm, MD, and Oliver Zolk, MD

Signals from pharmacovigilance studies indicate that women are at higher risk for adverse drug reactions (ADRs) due to diuretics. Despite the long-term use of torasemide, there are few studies investigating gender differences of torasemide pharmacokinetics in the hospital setting. Therefore, torasemide pharmacokinetics were investigated in 90 patients (45 women, 45 men) during steady-state conditions. Torasemide elimination was significantly reduced in women compared with men (eg, body-weight-normalized area under the concentration-time curve: 42.1 ± 20.4 vs 30.9 ± 10.3 kg•h/L; $P < .001$). Among the investigated genetic factors [SLC22A11(OAT4), SLCO1B1(OATP1B1), CYP2C9], only the SLCO1B1c.521T>C polymorphism had a

significant influence on torasemide pharmacokinetics. Using cell lines expressing OATP1B1, the authors identified torasemide as OATP1B1 substrate ($K_m = 6.2$ μ M) with a significant reduction of uptake by the 521C-variant. Taken together, gender differences in torasemide pharmacokinetics are likely to contribute to a higher rate of ADRs in women, which has, for example, been observed in a German Pharmacovigilance Project with 66% of hospitalizations due to torasemide ADRs occurring in women.

Keywords: Gender; torasemide; loop diuretics; OATP1B1
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Female patients are at higher risk for adverse drug reactions (ADRs) than male patients are.¹⁻⁶ A recent analysis of the German pharmacovigilance database, for example, demonstrated gender-specific

differences in the frequency of hospital admissions caused by ADRs secondary to the use of diuretics.^{7,8} In that study, two-thirds of serious ADRs related to diuretics occurred in women.^{7,8}

The underlying mechanisms for these observations have not been elucidated so far. Besides the concomitant use of medications, gender-specific differences in pharmacokinetics have been proposed.^{4,5} Indeed, in a recent pilot study, we identified gender as a potential determinant of torasemide pharmacokinetics.⁹

Pharmacokinetic studies performed in healthy volunteers suggest that torasemide is completely absorbed with little intersubject variation. Its metabolism and excretion, however, largely differ between individuals.

Extrarenal elimination accounts for about 80% of the total clearance of torasemide, partly through metabolism by the genetically polymorphic cytochrome P450 enzyme CYP2C9.⁹⁻¹³ Moreover, polymorphisms in the gene encoding the organic anion

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Table I Patient Characteristics

Parameter	Gender		<i>P</i>
	Female (n = 45)	Male (n = 45)	
Diagnosis (hypertension/heart failure)	23/22	22/23	
No. of all received drugs	8.0 ± 2.2	8.5 ± 2.3	.334 ^a
Age, y	72.1 ± 8.7	64.7 ± 9.4	<.001 ^a
BMI, kg/m ²	29.0 ± 5.1	27.4 ± 4.5	.039 ^a
Serum creatinine, μmol/L	75.3 ± 33.6	83.2 ± 36.4	.321 ^a
CL _{CR} , MDRD, mL/min/1.72 m ²	83.9 ± 47.2	95.0 ± 34.9	.035 ^a
<i>CYP2C9</i> c.230T allele frequency	0.133	0.144	.829 ^b
<i>CYP2C9</i> c.1075A allele frequency	0.078	0.056	.550 ^b
<i>SLCO1B1</i> c.388G allele frequency	0.378	0.356	.757 ^b
<i>SLCO1B1</i> c.521C allele frequency	0.200	0.156	.436 ^b
<i>SLC22A11</i> g.-20166T allele frequency	0.589	0.567	.975 ^b

BMI, body mass index; CL_{CR}, creatinine clearance. Number of all received drugs, age, BMI, serum creatinine, and CL_{CR} are given as mean ± SD. Bold font indicates significant p-values.

a. Mann-Whitney *U* test.

b. Pearson chi-square.

transporting polypeptide OATP1B1 (*SLCO1B1*), which mediates the hepatocellular uptake of drugs, have been reported to influence torasemide disposition.^{9,14}

Because torasemide is extensively bound to plasma protein (>99%), very little of the drug enters urine via glomerular filtration. Most of its renal clearance (approximately 20% of total clearance) occurs via active secretion by the proximal tubules into urine. Interindividual differences in renal clearance of torasemide have been partially explained by genetic variation in the luminally expressed organic anion transporter OAT4 (*SLC22A11*).^{14,15}

The objective of the present study was to quantify the impact of gender on torasemide pharmacokinetics during steady state in the hospital setting. Studies on determinants of torasemide pharmacokinetics in patients during steady-state treatment are largely missing. To address this issue, this study was performed in a relatively large cohort of patients on stable medication with torasemide for treatment of arterial hypertension and/or symptomatic heart failure (New York Heart Association [NYHA] Class II-III). To assess the true gender-specific impact on the intersubject variation of torasemide pharmacokinetics, we also considered as potential covariates demographic factors (eg, body weight, kidney function) as well as single-nucleotide polymorphisms (SNPs) in the *CYP2C9* (c.430C>T and c.1075A>C intermediate/poor-metabolizer genotypes), *SLCO1B1* (c.388A>G and c.521T>C), and *SLC22A11* (g.-20166A>T) genes, which in concert have been suggested to explain almost 50% variation

in total oral torasemide clearance.¹⁴ Moreover, by means of in vitro studies, we tested the hypothesis whether torasemide is indeed an OATP1B1 substrate and whether cells expressing the 521C variant have an impaired torasemide uptake.

METHODS

Study Design

An open-label pharmacokinetic study was performed in 90 patients with a primary diagnosis of arterial hypertension (n = 45) or NYHA Class II or III congestive heart failure (n = 45). Pharmacokinetic data of 24 of these patients have previously been reported.⁹ The study was approved by the Ethics Committee of the Federal State of Mecklenburg–West Pomerania. Criteria for inclusion were stable therapy with the diuretic torasemide for at least 1 week. All patients received 10 mg of torasemide once daily. Only 1 of the included subjects took 20 mg of torasemide once daily for clinical reasons. After written informed consent was obtained, all patients underwent a screening evaluation, which included laboratory tests. None of the patients had a history of additional liver disease or intrinsic kidney disease. Table I summarizes the patients' characteristics including serum creatinine and creatinine clearance. All long-term medications (see Table II) were maintained at a constant dose during the entire study phase of 1 week. Blood samples were collected on the study day before and at 0.5, 1, 2, 4, 8, 12, and 24 hours after torasemide administration in all patients.

Table II Prescription Frequency of Comedications in Male and Female Patients^a

Medication	Women (n)	Men (n)
Acetylsalicylic acid	27	36
Allopurinol	7	5
Amlodipine	5	6
Atorvastatin	8	13
Bisoprolol	26	26
Candesartan	8	3
Clopidogrel	11	8
Digitoxin	16	9
Dipyrrone	5	4
Felodipine	10	19
Levothyroxine	7	0
Metformin	4	4
Metoprolol	2	6
Moxonidine	11	2
Pantoprazole	31	34
Phenprocoumon	8	2
Quinapril	10	6
Ramipril	13	15
Simvastatin	19	27
Sultamicillin	2	11
Verapamil	7	2

a. Listed are all drugs prescribed in more than 5 patients.

In Vitro Uptake Studies

For uptake experiments, HEK293 cells (700 000 per well) were seeded in 12-well plates coated with 0.1 mg/mL poly-D-lysine. The cells were grown to confluence for 2 days and then incubated with 10 mmol/L sodium butyrate (Merck, Darmstadt, Germany) for 24 hours before the uptake experiments to stimulate higher expression of the recombinant proteins.¹⁶ Before starting the uptake experiments, the cells were washed with prewarmed (37°C) uptake buffer (142 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L K₂HPO₄, 1.2 mmol/L MgSO₄, 1.5 mmol/L CaCl₂, 5 mmol/L glucose, and 12.5 mmol/L N-2-hydroxyl piperazine-N'-2-ethane sulfonic acid, pH 7.3). Cells were then incubated with the uptake buffer containing torasemide at 37°C for 10 minutes. Subsequently, the cells were washed with ice-cold uptake buffer before lysing the cells with 0.2% sodium dodecyl sulfate. Finally, the intracellular accumulation of torasemide was determined by a validated liquid chromatography–tandem mass spectrometry (LC/MS/MS) assay (see below). Each experiment was repeated at least 3 times.

Site-Directed Mutagenesis

Both mutations (c.388A>G and c.521T>C) were introduced into the vector pcDNA3.1-OATP1B1 by using the QuikChange site-directed Mutagenesis kit from Stratagene (Amsterdam, the Netherlands) as previously described.¹⁷

Transport Assays in Transiently Transfected Cells

For the analysis of the effect of the mutations on the transport of torasemide, we used transiently transfected HEK293 cells expressing the respective OATP1B1 variant. On day 1, 300 000 parental HEK293 cells/well were seeded in 12-well plates. On day 2, the cells were transfected with the respective plasmid [pcDNA3.1-OATP1B1, pcDNA3.1-OATP1B1 c.388A>G, pcDNA3.1-OATP1B1 c.521T>C, and pcDNA3.1(+) serving as control, 1.5 µg DNA/well] by lipofection (Invitrogen, Groningen, the Netherlands) according to the manufacturer's protocol. On day 3, the cells were induced with 10 mmol/L sodium butyrate. Uptake assays were performed on day 4 as described above. To ensure sufficient protein expression, uptake of the prototypical OATP1B1 substrate [³H]bromosulphophthalein (Hartmann Analytic, Braunschweig, Germany) was routinely measured, showing uptake ratios between OATP1B1-expressing cells and vector-transfected control cells of 36-fold for reference OATP1B1, 33-fold for OATP1B1 c.388A>G, and 27-fold for OATP1B1 c.521T>C.

LC/MS/MS Assay for Torasemide

Samples were prepared by adding 100 µL of internal standard solution (clopamide 10 ng/L in acetonitrile), 50 µL 1 M HCl, and 4 mL n-hexane/diethylether (50%/50%, v/v) to 500 µL of plasma. After incubation for 15 minutes and centrifugation, 2 mL of the organic layer was transferred into glass tubes and dried under a stream of nitrogen, before reconstitution in 500 µL of acetonitrile and 2% acetic acid (90:10, v/v). The injected volume was 2 µL. The high-performance liquid chromatography (HPLC) system was an Agilent Series 1100 (Agilent Technologies Deutschland GmbH, Böblingen, Germany). An 8/3 Nucleosil 300-5 C₄ (Macherey-Nagel, Düren, Germany) HPLC column was used. A mixture of acetonitrile and 2% acetic acid was used as the mobile phase (90:10, v/v). The flow rate was set at 0.2 mL/min. The retention time of torasemide was 1.2 minutes, and of the internal standard, 0.5 minutes.

Quantification was performed with a Sciex API 4000 (Applied Biosystems, Toronto, Canada) triple quadrupole mass spectrometer (MS) equipped with an atmospheric pressure ionization using multiple-reaction monitoring and the positive ion mode. The main MS parameters were Q1, 349 m/z; Q3, 264 m/z; collision gas, 5 psi; curtain gas, 10 psi; ion spray voltage, 4500 V; and temperature, 700°C. The peak area ratio of torasemide to the internal standard was calculated using Analyst 1.4.2 software (Applied Biosystems). The lower limit of quantification was 1.0 µg/L. A calibration curve was constructed using 1/X-weighted linear regression between spiked plasma concentrations and the measured ratios. The calibration curves were linear over the range 1 to 1000 µg/L, with correlation coefficients >0.998. Plasma calibration standards (1, 2.5, 5, 10, 100, and 1000 µg/L), quality controls, and blank and double-blank samples were prepared in the same manner. The interday coefficient of variation was 10.3% at 1 µg/L, 7.0% at 100 µg/L, and 6.1% at 1000 µg/L ($n = 3 \times 5$).

Pharmacokinetic Analysis

Plasma concentration-time curves of torasemide were evaluated by noncompartmental analysis using WinNonlin Version 3.3 (Pharsight, Mountain View, California). Peak plasma concentration ($C_{\max}$) and time to reach peak plasma concentration ($t_{\max}$) were obtained directly from the observed data. Half-life ($t_{1/2}$) was calculated as $\ln 2 / \lambda_z$, where λ_z denotes the time constant of the terminal slope. The area under the plasma concentration-time curve during 24-hour dosing interval at steady state (AUC_{24}) was calculated using the linear trapezoidal rule. Oral clearances (CL_{oral}) were calculated as dose / AUC_{24} .

Genotyping

Genomic DNA was extracted from 200 µL of peripheral blood anticoagulated with ethylenediaminetetraacetic acid with the Flexigene Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instruction. Genotyping for the *CYP2C9* c.430C>T (*CYP2C9**2), *CYP2C9* c.1075A>C (*CYP2C9**3), *SLCO1B1* c.388A>G, *SLCO1B1* c.521T>C, and *SLC22A11* g-20166A>T SNPs was carried out using the TaqMan Pre-Developed Assay Reagents for Allelic Discrimination (Applied Biosystems, Weiterstadt, Germany) as described previously.⁹ Amplification was performed in a final volume of 5 µL containing 5 ng DNA, 4.5 pmol of each primer, 1.0 pmol of each probe, and 2.5 µL 2x TaqMan Genotyping Master Mix (contains PCR buffer, passive

reference dye ROX, deoxynucleotides, and AmpliTaq Gold DNA polymerase; Applied Biosystems) by use of the ABI Prism Sequence Detector 7900 (Applied Biosystems). Cycle parameters were as follows: 95°C for 10 minutes and then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. After PCR, fluorescence yield for the 2 different dyes was measured. We used the SDS 2.1 software (Applied Biosystems) to plot and automatically call genotypes on the basis of a 2-parameter plot with fluorescence intensities of FAM and VIC.

Statistics

The results are expressed as mean values $\pm$ standard deviation in the text and tables and as mean values $\pm$ SEM in the figures. Multiple regression analysis (after adjustment by age, body mass index [BMI], and creatinine clearance) was used to assess the impact of gender and polymorphisms on pharmacokinetic parameters. SNPs were analyzed in a dominant model. For each SNP, 2 dummy variables were defined for the homozygous reference genotype and genotypes with at least 1 variant allele. Statistical comparison of pharmacokinetic parameters of male versus female patients was done by use of the Mann-Whitney *U*-test. Allele frequencies in female and male patients were compared with the Pearson chi-square test. Nonparametric comparisons of more than 2 groups were performed with the Kruskal-Wallis tests. Two-way analysis of variance was used for statistical analysis of concentration-uptake curves. Data were analyzed with SPSS 16.0 for Windows (SPSS Inc, Chicago, Illinois). Statistical significance was accepted for values of $P < .05$.

RESULTS

Before implementation of this study, a statistical power analysis was performed to assess the required number of patients for identifying an association between gender and steady-state torasemide pharmacokinetics. Based on a pilot study, which demonstrated 1.52-fold higher weight-normalized AUC_{24}/D values in women compared with men, a sample size of 45 male and 45 female patients can provide a statistical power of 99% and 98% at the nominal type I error rate of 0.05 and 0.01, respectively.

Patients' characteristics are summarized in Table I. Significant differences were observed for age, BMI, and the modification of diet in renal disease (MDRD) equation-estimated glomerular filtration rate (clearance of endogenous creatinine, CL_{CR}), whereas serum

Table III Torasemide Pharmacokinetic Parameters Stratified for Gender

Parameter	Gender		<i>P</i>
	Female (n = 45)	Male (n = 45)	
AUC _{24h} /D, µg·h/L/mg	570 ± 283	382 ± 143	<.001
Weight-normalized AUC ₂₄ /D, kg·h/L	42.1 ± 20.4	30.9 ± 10.3	<.001
C _{max} /D, µg/L/mg	129.6 ± 57.0	97.5 ± 40.7	.001
t _{max} , h	1 (0.5-4)	1 (0.5-4)	.373
t _{1/2} , h	4.3 ± 1.2	3.6 ± 1.0	.003
CL _{oral} , L/h	2.1 ± 0.8	3.0 ± 1.1	<.001

AUC_{24h}/D, weight-normalized AUC₂₄/D; D, dose. C_{max}/D, t_{1/2}, and CL_{oral} are given as mean ± SD. t_{max} is given as the median and range. *P*, Mann-Whitney *U*. Bold font indicates significant *p*-values.

Figure 1. Mean ± SEM steady-state torasemide plasma concentration-time course in male and female patients with arterial hypertension or heart failure treated with torasemide. Insert: Weight-normalized torasemide AUC₂₄/D values stratified for gender. Horizontal lines show the geometric mean of each group (*P* = .0004 male vs female, Mann-Whitney *U*-test).

creatinine was similar between male and female patients. Comedication included a wide spectrum of different drugs, as listed in Table II. The average number of compounds prescribed to male or female patients was not significantly different (8.5 vs 8.0). The comedication did not include prototypical CYP2C9 inhibitors, such as amiodarone and fluconazole, or OATP inhibitors, such as rifampicin and gemfibrozil. One female patient, however, was treated with cyclosporine A, a known OATP1B1 inhibitor.^{18,19} This patient displayed the highest torasemide AUC₂₄ value, which was not explained by the presence of a nonfunctional *SLCO1B1* c.388G or c.521C allele.

Frequencies of the polymorphic *CYP2C9* c.230T, *CYP2C9* c.1075A, *SLCO1B1* c.388G, *SLCO1B1* c.521C, and *SLC22A11* g.-20166T alleles did not differ between men and women and did not

Figure 2. Gender-specific comparison of age- and weight-normalized torasemide AUC₂₄/D (statistical comparisons with Mann-Whitney *U*-test). Box plots are summarizing the following statistical measures: median, upper and lower quartiles, and minimum and maximum data values. Outliers (*, patient treated with cyclosporine A) and single values (+) are shown separately. The numbers of patients are indicated under each box.

significantly (*P* > 0.3) deviate from the Hardy-Weinberg equilibrium.

A high interindividual variability of torasemide pharmacokinetics was observed in our study population. For instance, torasemide dose- and weight-normalized AUC₂₄ and C_{max} values differed 14.5-fold (Figure 1; coefficient of variation [CV] = 51.1%) and 10.3-fold (CV = 45.7%), respectively, between individual patients. Plasma concentrations of torasemide were on average higher in women compared with men (Table III, Figure 1). Steady-state pharmacokinetic parameters of torasemide, stratified for gender, are summarized in Table III. Significant differences between male and female patients were found in all pharmacokinetic parameters except for t_{max}. The data indicate about 30% to 40% higher mean AUC₂₄ and C_{max} values in female patients compared with male patients. This increase in AUC was observed irrespective of the age of the patients, as shown in Figure 2.

Table IV Multiple Linear Regression Analysis of the Effect of Gender and *CYP2C9*, *SLCO1B1*, and *SLC22A11* Polymorphisms on Torasemide Pharmacokinetic Parameters

Parameter ^a	B	CI	P
Weight-normalized AUC ₂₄ /D, kg·h/L			
Gender	9.4	1.0 to 17.9	.029
<i>CYP2C9</i> c.430C>T	3.8	-4.6 to 12.1	.374
<i>CYP2C9</i> c.1075C>A	4.9	-5.8 to 15.7	.366
<i>SLCO1B1</i> c.388A>G	-1.7	-14.1 to 10.6	.783
<i>SLCO1B1</i> c.521T>C	8.3	0.5 to 16.1	.037
<i>SLC22A11</i> g.-20166A>T	2.4	-7.6 to 12.3	.639
C _{max} /D, µg/L/mg			
Gender	32	7 to 56	.011
<i>CYP2C9</i> c.430C>T	18	-6 to 45	.140
<i>CYP2C9</i> c.1075C>A	17	-14 to 48	.287
<i>SLCO1B1</i> c.388A>G	13	-23 to 49	.483
<i>SLCO1B1</i> c.521T>C	-8	-30 to 15	.508
<i>SLC22A11</i> g.-20166A>T	27	-2 to 56	.070
t _{1/2} , h			
Gender	0.4	-0.1 to 1.0	.143
<i>CYP2C9</i> c.430C>T	0.3	-0.2 to 0.9	.263
<i>CYP2C9</i> c.1075C>A	0.0	-0.7 to 0.7	.980
<i>SLCO1B1</i> c.388A>G	0.6	-0.3 to 1.4	.187
<i>SLCO1B1</i> c.521T>C	0.3	-0.2 to 0.8	.281
<i>SLC22A11</i> g.-20166A>T	0.3	-0.3 to 1.0	.308
CL _{oral} , L/h			
Gender	-0.8	-1.2 to -0.3	.001
<i>CYP2C9</i> c.430C>T	0.1	-0.3 to 0.5	.609
<i>CYP2C9</i> c.1075C>A	-0.4	-0.9 to 0.2	.211
<i>SLCO1B1</i> c.388A>G	0.0	-0.6 to 0.6	.957
<i>SLCO1B1</i> c.521T>C	-0.6	-1.0 to -0.3	.002
<i>SLC22A11</i> g.-20166A>T	-0.1	-0.6 to 0.4	.792

a. Adjusted by age, body mass index, and CL_{CR}. B, regression coefficient; CI, 95% confidence interval; D, dose. Bold font indicates $P < .05$.

We considered as potential covariates SNPs in the *CYP2C9* (c.430C>T and c.1075A>C), *SLCO1B1* (c.388A>G and c.521T>C), and *SLC22A11* (g.-20166A>T) genes, which in concert have been suggested to explain a large portion of the interindividual variability (eg, in total oral torasemide clearance).¹² Among the genetic factors investigated, only the *SLCO1B1* c.521T>C SNP had a significant independent effect on torasemide pharmacokinetics, as demonstrated by multiple regression analysis after adjustment of pharmacokinetic parameters for age, BMI, and CL_{CR} (Table IV). In this analysis, gender remained a strong independent determinant of AUC₀₋₂₄/D, C_{max}/D, and CL_{oral}. After covariate adjustment, mean weight-normalized AUC₂₄/D was significantly higher, and mean CL_{oral} was significantly lower in female patients compared with male patients (Table IV). Figure 3 shows combined effects of gender and *SLCO1B1* c.521T>C

genotypes on the plasma concentration-time curve, AUC₂₄/D, and CL_{oral} of torasemide. Mean oral clearance of torasemide was highest in male patients with the *SLCO1B1* c.521TT genotype (3.1 ± 1.2 L/h), whereas it was 42% lower in female patients with the TC or CC genotype (1.8 ± 0.5 L/h, $P < .0001$). Accordingly, mean AUC₂₄ values were lowest in men homozygous for the *SLCO1B1* c.521 wild-type allele (AUC₂₄, weight- and dose-normalized, 28.3 ± 6.7 kg·h/L) and increased by 63% in women carrying at least 1 *SLCO1B1* c.521 variant allele (46.1 ± 15.6 kg·h/L, $P < .001$). Similar results were obtained when the patient, who was treated with the known OATP1B1 inhibitor cyclosporine A, was excluded from the analysis.

To investigate the impact of *SLCO1B1* SNPs on OATP1B1-mediated uptake of torasemide, in vitro experiments were performed in HEK cells transfected to express either the wild-type²⁰ or the mutant

Figure 3. (A) Mean $\pm$ SEM steady-state torasemide plasma concentration-time course in patients with arterial hypertension or heart failure treated with torasemide. Groups were stratified according to gender and OATP1B1 c.521T>C. (B) Scatterplots of weight-normalized AUC₂₄/D and oral clearance of torasemide. Values are shown for each individual patient stratified by gender and OATP1B1 c.521T>C. Horizontal lines indicate the geometric mean of each group (statistical comparisons with Kruskal-Wallis tests).

transporters.¹⁷ Compared with cells transfected with the expression vector only, recombinant expression of OATP1B1 significantly enhanced uptake of torasemide (2.5 ± 0.1 vs 5.4 ± 0.2 ng torasemide/mg protein/10 min, $P < .01$, $n = 6$). Figure 4A shows the effects of increasing concentrations of torasemide on the rate of OATP1B1-mediated uptake. The apparent K_m was 6.2 ± 3.3 μ M, and V_{max} was 12.8 ± 2.6 pmol/mg/min. The c.521T>C, but not the c.388A>G, variant (Figure 4B) showed a significantly reduced transport of torasemide (1.7 ± 1.9 and 6.6 ± 1.7 pmol/mg/min, respectively, at 10 μ M torasemide) compared with wild-type OATP1B1 (6.0 ± 2.7 pmol/mg/min).

Figure 4. (A) Kinetics of torasemide uptake by wild-type OATP1B1. Data were fit to the Michaelis-Menten equation after subtracting background uptake in cells transfected with the vector only. Data points represent the mean $\pm$ SEM from 3 independent experiments. (B) Uptake of torasemide into transiently transfected (1.5 μ g plasmid DNA/300,000 cells) HEK293 cells expressing human OATP1B1 (wild-type), OATP1B1 p.130Asn>Asp (SLCO1B1 c.388A>G), and OATP1B1 p.174Val>Ala (SLCO1B1 c.521T>C). Data are shown as mean $\pm$ SEM of 3 uptake experiments ($P < .05$ wild-type vs c.521T>C, 2-way analysis of variance).

DISCUSSION

Clinical data on gender-related differences with regard to adverse or therapeutic effects of diuretics are largely missing, although animal studies support the notion that such differences may exist. Cerrutti et al,²¹ for example, demonstrated a significantly lower renal clearance of furosemide in female rats. Furosemide as well as torasemide induced diuresis, natriuresis, and kaliuresis more effectively in female rats than in males.^{22,23}

A recent analysis from German national pharmacovigilance centers indicated that hospitalizations due to ADRs caused by diuretics are more frequent

in women than in men.^{7,8} The more frequent incidence of ADRs in women was obvious for all commonly prescribed diuretics including torasemide (29 reports about serious ADRs in men vs 57 in women) and was not explained by gender-specific differences in drug prescription rates.^{7,8} The goal of the present study was to prospectively evaluate the impact of gender on steady-state pharmacokinetics of torasemide, which may at least in part explain the unbalanced distribution of ADRs among male and female gender.

The main result of this study is that gender as well as the *SLCO1B1* c.521T>C SNP are significant and independent predictors of steady-state pharmacokinetics of torasemide. Oral clearance of torasemide was on average one-third lower in women than in men, which was associated with elevated mean AUC_{24}/D and C_{max} values in women compared with men. These results also persisted after covariate adjustment for age, BMI, and MDRD equation-estimated glomerular filtration rate, which were not balanced between the male and the female study cohort. Altogether, our results indicate that gender is an independent factor that affects the pharmacokinetics of torasemide, providing a mechanism that may at least in part explain the increased toxicity of torasemide in women.

The proportion of pharmacokinetic variability explained by gender was greater than that explained by the presence of the *SLCO1B1* c.521C variant allele: 11% versus 7% for CL_{oral} and 11% versus 5% for weight-normalized AUC_{24}/D . Except for the *SLCO1B1* c.521T>C SNP, no other tested genetic covariates (*SLCO1B1* c.388A>G, *CYP2C9* c.430C>T, *CYP2C9* c.1075C>A, and *SLC22A11* g.-20166A>T SNPs) were consistently associated with torasemide pharmacokinetic parameters. Comparison of our results with a recent analysis¹⁴ suggests that although the relative contribution of genetic factors, such as *CYP2C9* or *OAT* SNPs, to torasemide pharmacokinetics is clearly evident in single-dose studies in young healthy volunteers, it is less significant in patients. In the clinical setting, gender, advanced age, disease state, comorbidities, or comedications may outweigh the relative contribution of genetic factors.

OATP1B1 is expressed in the liver and has been shown to be important to the hepatic uptake of a number of drugs in clinical use.²⁴⁻²⁶ Although clinical association studies consistently indicate that OATP1B1 might be involved in the clearance of torasemide, transport of torasemide by OATP1B1 has not been shown so far. Our in vitro experiments

for the first time directly showed uptake of torasemide into cells expressing OATP1B1 with a K_m value in the low micromolar range, demonstrating that torasemide is a substrate of OATP1B1. These results are consistent with an OATP1B1-mediated effect on torasemide pharmacokinetics mainly during drug absorption from the portal circulation.

The genetic basis for considerable variation of OATP1B1 activity has been described.^{24,25,27} Two common variants appear to have altered function: OATP1B1 p.130Asn>Asp (*SLCO1B1* c.388A>G) and OATP1B1 p.174Val>Ala (*SLCO1B1* c.521T>C). While reduced activity of OATP1B1 p.174Val>Ala for several substrates has been observed consistently, OATP1B1 p.130Asn>Asp showed reduced, unchanged, or increased activities depending on the substrate tested.^{24,25,27,28} In our study, reduced transport of torasemide was observed in cells expressing the c.521T>C variant but not in cells expressing the c.388A>G variant. These in vitro findings correspond to the observed association of the *SLCO1B1* c.521T>C but not the *SLCO1B1* c.388A>G SNP with torasemide pharmacokinetics. Reduced hepatocellular uptake of torasemide by the OATP1B1 p.174Val>Ala variant will result in higher plasma concentrations because of decreased hepatic metabolism.

In summary, our study demonstrates a significant impact of gender and confirms the impact of the *SLCO1B1* c.521T>C SNP on the steady-state pharmacokinetics of torasemide in the clinical setting in a representative cohort of patients. Our observations may at least in part explain the unbalanced distribution of torasemide ADRs among male and female gender.

CONCLUSIONS

For the first time, this study demonstrates a significant impact of gender on the pharmacokinetics of torasemide in a representative patient population. Furthermore, among the investigated genetic factors (*SLCO1B1* [encoding for OATP1B1; c.388A>G and c.521T>C], *CYP2C9* [c.430C>T and c.1075C>A], and *SLC22A11* [encoding for OAT4; g.-20166A>T]), only the *SLCO1B1* c.521T>C polymorphism had a significant influence on torasemide pharmacokinetics in the clinical setting. Accordingly, we could identify torasemide as an OATP1B1 substrate in vitro. Torasemide uptake in vitro is impaired in cells expressing the 521C variant, which explains the significantly higher plasma concentrations of torasemide in humans carrying the 521C allele.

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