

THE ANTI-INFLUENZA DRUG OSELTAMIVIR EXHIBITS LOW POTENTIAL TO INDUCE PHARMACOKINETIC DRUG INTERACTIONS VIA RENAL SECRETION—CORRELATION OF IN VIVO AND IN VITRO STUDIES

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ABSTRACT:

Osetamivir is an ester prodrug of the active metabolite [3*R*,4*R*,5*S*]-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate phosphate (Ro 64-0802), a potent and selective inhibitor of neuraminidase enzyme of influenza virus. Osetamivir is rapidly hydrolyzed by hepatic carboxylesterases to Ro 64-0802, which is then exclusively excreted by glomerular filtration and active tubular secretion without further metabolism. In vivo and in vitro studies were conducted to evaluate the renal drug-drug interaction potential of osetamivir. Crossover studies were conducted in healthy subjects in which oral osetamivir was administered alone and coadministered with probenecid, cimetidine, or amoxicillin. Probenecid completely blocked the renal secretion of Ro 64-0802, increasing systemic exposure (area under the curve) by 2.5-fold, but no interaction was observed with cimetidine or amoxicillin. These in vivo data show that Ro 64-0802 is

secreted via an organic anion pathway, but Ro 64-0802 does not inhibit amoxicillin renal secretion. In vitro effects of Ro 64-0802 on the human renal organic anionic transporter 1 (hOAT1) were investigated using novel Chinese hamster ovary cells stably transfected with hOAT1. Ro 64-0802 was found to be a low-efficiency substrate for hOAT1 and a very weak inhibitor of hOAT1-mediated transport of *p*-aminohippuric acid (PAH). Ro 64-0802 did not inhibit the hOAT1-mediated transport of amoxicillin. In contrast, probenecid effectively inhibited the transport of PAH, Ro 64-0802, and amoxicillin via hOAT1. These in vitro observations are consistent with the in vivo data, validating the usefulness of the in vitro system for evaluating such drug-drug interaction. The study results demonstrate that osetamivir has a low drug-drug interaction potential at the renal tubular level due to inhibition of hOAT1.

Osetamivir is an orally bioavailable prodrug of Ro 64-0802¹ (GS4071), a potent and selective inhibitor of influenza A and B neuraminidase (Bardsley-Elliott and Noble, 1999). After oral administration, the prodrug is extensively hydrolyzed to its active metabolite Ro 64-0802, which is then extensively excreted by glomerular filtration and renal tubular secretion without further metabolism (He et al., 1999a).

Active renal secretion occurs via specific transport proteins located in the basolateral and apical membrane of the proximal tubule (Pritchard and Miller, 1993). Two general drug secretion pathways exist in proximal tubules, one for basic compounds (organic cation transport system) and another for acidic compounds (organic anion transport system). Thus far, two active pathways capable of transporting or-

ganic cations have been identified in kidney, OCT1 and OCT2 (Grundemann et al., 1994; Okuda et al., 1996). Similarly, several renal organic anion transporters have been recently cloned and characterized. Of these, organic anion transporter 1 (OAT1) is the main component mediating tubular secretion of organic acids (Sekine, 1997; Sweet et al., 1997). It is located in the basolateral membrane of proximal tubules (Tojo et al., 1999) and exhibits a broad substrate specificity [e.g., β -lactam antibiotics (Jariyawat et al., 1999), antiviral nucleotide analogs (Cihlar et al., 1999), nonpeptidic angiotensin inhibitors (Edwards et al., 1999), and nonsteroidal anti-inflammatory drugs (Apiwattanukul et al., 1999)]. Recently, a human ortholog of OAT1 (hOAT1; hPAH) has been cloned and characterized (Hosoyamada et al., 1999; Lu et al., 1999; Cihlar et al., 1999). In addition, expression of several other organic anion transporters have been localized to human kidney [e.g., OATP (Kullak-Ublick, 1995), hOAT3 (Cha et al., 2001; Takeda et al., 2001), and hOAT4 (Cha et al., 2000)]. Unlike hOAT3, which is present in basolateral membrane of proximal tubules and thus mediates tubular excretion of organic anions (Cha et al., 2001), precise localization and function of hOAT4 and OATP in human kidney has yet to be determined.

To identify the renal secretion pathway for a particular drug, in vivo pharmacokinetic drug interaction studies are usually conducted with probe drugs, such as probenecid and cimetidine. Probenecid, a potent inhibitor of hOAT1 (Ho et al., 2000), is known to competitively inhibit the secretion of many weak organic acids. In contrast, cime-

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¹ Abbreviations used are: Ro 64-0802, [3*R*,4*R*,5*S*]-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate phosphate; OCT, organic cation transport; OAT, organic anion transporter; hOAT, human renal organic anion transporter; OATP, organic anion transporter polypeptide; PAH, *p*-aminohippuric acid; AUC, area under the curve; CHO^{hOAT}, Chinese hamster ovary cells transfected to express functional hOAT1; CHO^{PIRES}, control cells transfected with the pIRESneo expression vector; CL_R, renal clearance.

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tidine, an efficient inhibitor of OCT1 and OCT2 (Urakami et al., 1998), is known to compete for active tubular secretion primarily with basic drugs. In addition, cimetidine has recently been identified as a potent inhibitor of the hOAT3-mediated transport of organic anions (Cha et al., 2001).

Coadministration of two drugs interacting with the same renal transporter may lead to the inhibition of active secretion of one or both drugs. Although animal pharmacokinetic studies may provide some insight into the specific drug-drug interactions, their results should be interpreted cautiously because of interspecies difference in organ transport pathways (Dresser et al., 2000) and may require clinical studies for confirmation. It is therefore desirable to develop an *in vitro* screening system that could provide a quick assessment of potential pharmacokinetic drug interactions subsequent to interference with specific secretion pathways.

A cell line stably expressing functional hOAT1 is currently being explored as an *in vitro* model to evaluate the interaction of various drugs with hOAT1 (Lin et al., 1999; Cihlar and Ho, 2000; Ho et al., 2000; Mulato et al., 2000). In this model, hOAT1 mediates transport of *p*-aminohippuric acid (PAH), a prototypic substrate for the renal organic anion transport system, with a K_m of 12.3 μM (Ho et al., 2000). Importantly, hOAT1 transport activity is sensitive to probenecid and can be stimulated by preloading the cells with glutarate, indicating that the heterogeneously expressed hOAT1 functions similarly to the native transporter (Ho et al., 2000). Some of the β -lactam antibiotics (e.g., cephaloridine, cephaloglycin, and cephalothin) (Lin et al., 1999) and nonsteroidal anti-inflammatory drugs (Mulato et al., 2000), which have been shown *in vivo* to interact with the renal transport of organic anions, efficiently inhibit hOAT1 transport activity in this system.

A clinical drug interaction study with probenecid and cimetidine was conducted to determine the pathway of Ro 64-0802 secretion. Another study was undertaken to investigate whether Ro 64-0802 can affect the pharmacokinetics of other drugs that are excreted via the anionic renal tubular secretion. Amoxicillin was selected as a suitable test drug since it is primarily eliminated by this pathway (Staniforth et al., 1983) and is an antibiotic commonly used in the treatment of respiratory infections, including secondary infections associated with influenza. Finally, we used a novel *in vitro* cell-based assay to characterize the interactions of Ro 64-0802 with hOAT1 and to address the potential interference of Ro 64-0802 with the hOAT1-mediated transport of amoxicillin. Evaluating the consistency between the *in vivo* pharmacokinetic studies and the *in vitro* hOAT1 transport experiments could determine whether this *in vitro* model can provide a suitable screening system to evaluate renal drug interaction potential of oseltamivir and other renally secreted drugs.

Materials and Methods

Clinical Study Drugs. Two separate drug interaction studies were conducted. In study 1, oseltamivir was administered alone and coadministered with probenecid or cimetidine, and in study 2, oseltamivir was administered alone and coadministered with amoxicillin. In study 1, the drugs obtained from Roche Clinical Trial Supplies (Basel, Switzerland) consisted of 75 mg of oseltamivir capsules, cimetidine 400-mg tablets, and Benemid (500 mg of probenecid) tablets. Cimetidine tablets were obtained from Alpharma Limited (Barnstaple, Devon, UK), and Benemid tablets were obtained from AAH Pharmaceuticals (Cheshire, UK). In study 2, the drugs supplied consisted of 75 mg of oseltamivir capsules and Clamoxyl (500 mg of amoxicillin) capsules.

Study Subjects/Ethics/Screening/Inclusion/Exclusion Criteria. Normal healthy male and female subjects aged 18 to 65 were enrolled. Both studies were conducted in full conformance with the principles of the "Declaration of Helsinki" and "Good Clinical Practice". No concomitant medication was permitted except for medication to treat adverse effects; however, study 2

allowed hormone replacement therapy and oral contraceptives. Written informed consent was obtained from each subject before screening, which included a brief medical history, physical examination, 12-lead ECG, semisupine blood pressure, pulse rate, oral temperature, and body weight measurement. Blood and urine samples were taken for laboratory safety tests, including a test for drugs of abuse and a urine pregnancy test for women of childbearing potential. Subjects with evidence of clinically relevant cardiovascular, endocrine, hematological, psychiatric, neurological, gastrointestinal, renal, hepatic, pulmonary or allergic disorders, or with a relevant history of drug or alcohol dependence were excluded.

Study Dosage/Design/Sampling. *Study 1.* This was a single-center, open-label, three-way randomized crossover study in which 18 subjects (nine male and nine female) were assigned to one of six treatment sequences (ABC, ACB, BAC, BCA, CAB, CBA). Treatment A consisted of a single 150-mg dose of oseltamivir administered alone. Treatment B consisted of a single 150-mg dose of oseltamivir, administered during treatment with cimetidine, in which 400 mg of cimetidine was given every 6 h for 16 doses beginning 23 h before the dose of oseltamivir. Treatment C consisted of a single 150-mg dose of oseltamivir, administered during treatment with probenecid, in which 500 mg of probenecid was given every 6 h for 16 doses beginning 23 h before the dose of oseltamivir. There was a 9 to 12 day washout period between treatments. All doses of oseltamivir, cimetidine, and probenecid were taken with 150 ml of water. Venous blood samples (approximately 7.5 ml) were collected via a cannula into Monovettes containing EDTA as an anticoagulant. Blood-sampling times for pharmacokinetic assessment were scheduled from 0.5 h before oseltamivir dose and up to 72 h after dosing. Each blood sample was kept on crushed ice until centrifugation (1500g/3000 rpm at 4°C for 10 min). The plasma supernatant was transferred immediately into a plain plastic tube, which was stored at -20°C until assay. Urine samples were collected at specified intervals until 72 h after oseltamivir dosing. The volume of urine output at each collection period was measured, and the pH of each bulk sample was determined. Two aliquots (a total of 30 ml) of the urine sample were stored frozen until analysis.

Study 2. This was a single-center, open-label, three-way randomized crossover study in which 12 subjects (six male and six female) were placed in one of two treatment sequences consisting of three treatments (ABC or BCA). Treatment A consisted of a single dose of 500 mg of amoxicillin dosed in the morning. Treatment B consisted of 75 mg of oseltamivir administered b.i.d. (every 12 h, morning and evening) for 4 days. Treatment C consisted of single doses of 75 mg of oseltamivir and 500 mg of amoxicillin administered together on the morning of the day immediately following treatment B. Each subject received a total of two doses of 500 mg of amoxicillin and nine doses of 75 mg of oseltamivir (75 mg b.i.d. for 4 days plus one single 75-mg dose on the following day). There was a washout period of 3 days between the single dose (treatment A) and multiple dose (treatments B/C) phases of the study. On pharmacokinetic days, serial blood samples (5 ml each) were collected from each patient via a cannula into a plastic tube/Monovette/Vacutainer containing EDTA as an anticoagulant, before dosing and until 12 h after dosing. After centrifugation, the plasma supernatant was split into two aliquots for the Ro 64-0802 and the amoxicillin assay. Urine was collected up to 60 min before dosing and through 12 h after dosing. The volume of urine output and pH at each of the postdose collection periods was measured. Urine aliquots (20 ml each) were frozen at -20°C until assay. One aliquot was used for the Ro 64-0802 assay and the second for the amoxicillin assay.

Drug Analysis in Plasma and Urine. Plasma and urine concentrations of oseltamivir and the active metabolite Ro 64-0802 were determined by a sensitive and specific high-performance liquid chromatography/tandem mass spectrometry method (Wiltshire et al., 2000). The limit of quantitation for Ro 64-0802 in plasma was 10 ng/ml, and in urine, it was 30 ng/ml. Cimetidine and probenecid concentrations were not assayed. Plasma concentrations of amoxicillin were determined by a sensitive and specific liquid chromatography/UV method, and urine concentrations were determined by a sensitive and specific liquid chromatography/tandem mass spectrometry method. The limit of quantitation for amoxicillin was 200 ng/ml in plasma and 100 ng/ml in urine.

Pharmacokinetic/Statistical Analysis. Model-independent pharmacokinetic parameters were estimated from individual plasma concentration-time and urinary recovery data using Microsoft Excel (Redmond, WA). The following parameters were calculated whenever appropriate: C_{max} (the maximum

observed plasma concentration), t_{max} (time to maximum observed plasma concentration), AUC_{0-12} (the area under the plasma concentration-time curve from 0–12 h, computed by the linear trapezoidal rule), AUC_{last} (the area under the plasma concentration-time curve from 0 to t_{last} , where t_{last} is the time of the last measurable concentration), AUC_{∞} (the total area under the plasma concentration-time curve extrapolated to infinity, calculated as $AUC_{last} + C_{last}/\lambda_z$, where C_{last} is the last measurable concentration and λ_z is the terminal elimination rate constant), f_e (the percentage of drug excreted into the urine), and CL_R (the renal clearance, computed as the ratio of the amount of drug excreted into urine to the equivalent area under the plasma concentration-time curve).

Ro 64-0802- and amoxicillin-computed pharmacokinetic parameters were listed and summarized by regimen. Confidence intervals (90%) for the ratio of the means (log transformed) in the presence and absence of concomitant drug were computed for comparison to the $\pm 20\%$ bioequivalency range. All statistical analyses were done using the SAS procedure PROC GLM (SAS, Cary, NC).

Cells and Reagents for In Vitro Studies. In vitro experiments were carried out using Chinese hamster ovary cells stably transfected to express functional hOAT1 (CHO^{hOAT}) and corresponding control cells transfected with the pIRESneo expression vector (CHO^{pIRES}). Generation of both cell lines and their characterization is described elsewhere (Ho et al., 2000). CHO^{hOAT} cells represent a cellular clone with hOAT1 transport activity isolated from a pool of stable transformants. The cells were maintained in phenol red-free F-12 nutrient mixture (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and 1 mg/ml G-418. The cells used in the transport experiments were grown in the absence of G-418. [³H]p-aminohippuric acid (4.08 Ci/mmol) was obtained from PerkinElmer Life Sciences (Boston, MA). [³H]Amoxicillin (11 Ci/mmol), [¹⁴C]Ro 64-0802 (40 mCi/mmol), and [³H]Ro 64-0802 (8.0 Ci/mmol) were prepared by Roche Discovery (Welwyn, UK). All other reagents were purchased from Sigma (St. Louis, MO) at the highest purity available.

Transport Assays. The transport assays were carried out in 12-well plates with nearly confluent CHO^{hOAT} and control CHO^{pIRES} cells seeded 48 h before each experiment (Ho et al., 2000). Immediately before the experiment, the cells were washed with phosphate-buffered saline. Waymouth buffer (135 mM NaCl, 5 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgCl₂, 0.8 mM MgSO₄, 28 mM glucose, and 13 mM HEPES, pH 7.2; 450 μ l/well) containing radiolabeled substrate was added to the cells and incubated for 30 min at 37°C. At the end of incubation, the cells were washed 3 times with ice-cold phosphate-buffered saline (2 ml/well) and lysed directly on the plate in the presence of 0.4% Triton X-100 lysis buffer (0.5 ml/well) for 15 min. Subsequently, the wells were washed with an additional 0.5 ml of the lysis buffer, the lysate and wash were combined, and radioactivity in each sample was counted. The number of cells was determined in parallel samples, and the intracellular drug accumulation was expressed in picomoles per 10⁶ cell. Both CHO^{hOAT} and control CHO^{pIRES} cells were incubated with 10 μ M [³H]amoxicillin in the presence of 500 μ M probenecid or Ro 64-0802. Similarly, the effects on Ro 64-0802 transport were examined using 250 μ M [¹⁴C]Ro 64-0802 or [³H]Ro 64-0802 in the presence of 500 μ M probenecid or amoxicillin. Tritium-labeled Ro 64-0802/903 of high specific activity (8 Ci/mmol) was produced especially for this experiment.

Determination of Drug Inhibition Constants. To determine inhibition constants (K_i), CHO^{hOAT} cells were incubated with [³H]PAH (5–40 μ M) either alone or in the presence of probenecid (3 and 9 μ M), amoxicillin (6 and 10 mM), or Ro 64-0802 (3 and 15 mM). The incubations were carried out for 3 min, and the cells processed as stated above. The K_i values were estimated by linear regression from double reciprocal (Lineweaver-Burke) plots using Enzyme Kinetics software (ChemSW, Fairfield, CA).

Results

Subject Demography. In study 1, a total of 18 subjects (nine males, nine females) completed the study, with an average age of 34.8 years (range, 20–62) and average weight of 68.9 kg (range, 48–89). In study 2, a total of 12 subjects (six males, six females) completed the study, with an average age of 24.4 years (range, 18–35) and average weight of 67.9 kg (range, 54–91). All subjects were Caucasians, except one non-Caucasian subject in study 1.

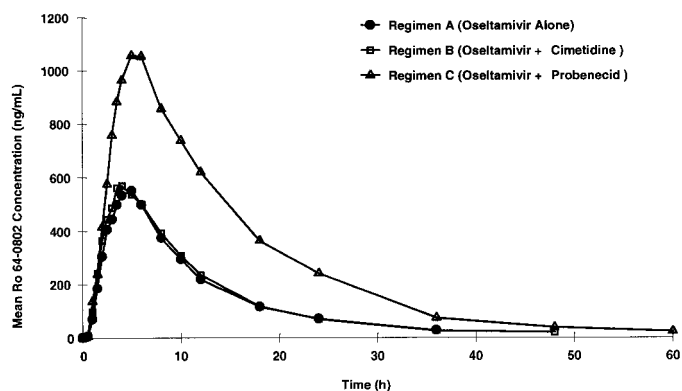


FIG. 1. Mean plasma concentrations of the active metabolite Ro 64-0802 following a single oral dose of oseltamivir (150 mg) given alone, with cimetidine (400 mg q.i.d.), or with probenecid (500 mg q.i.d.) to healthy volunteers ($n = 18$).

Effect of Probenecid and Cimetidine on Ro 64-0802 Renal Clearance. Mean plasma concentration-time profiles for Ro 64-0802 for the three regimens are shown in Fig. 1. Mean (S.D.) plasma pharmacokinetic parameters are summarized in Table 1. Urinary recovery, reported as a percentage of the administered dose over the 0- to 72-h collection period, and renal clearance are included in Table 1. As observed in Fig. 1 and Table 1, the mean parameter values, ratios, and 90% confidence intervals show an altered pharmacokinetic profile for Ro 64-0802 when regimen C (oseltamivir given during treatment with probenecid) is compared with regimen A (oseltamivir given alone), subsequent to a more than 50% decrease in renal clearance from 15.7 to 7.5 l/h. This reduced renal clearance resulted in an increase in total exposure (AUC_{∞}) of Ro 64-0802 by approximately 2.5-fold, and C_{max} increased by about 2-fold. As observed in Fig. 1 and Table 1, cimetidine had very little effect upon the pharmacokinetics of Ro 64-0802. The renal clearance was not altered, and mean values for the other pharmacokinetic parameters were very similar between regimen A (oseltamivir given alone) and regimen B (oseltamivir given during treatment with cimetidine). The 90% confidence intervals for the ratio of the means for C_{max} were within the 80 to 125% bioequivalence criteria, whereas those for AUC_{∞} were only marginally outside the 80 to 125% window.

Osetamivir—Amoxicillin Interaction Study. The mean (S.D.) values and 90% confidence intervals of the primary pharmacokinetic parameters for amoxicillin are summarized in Table 2, and the mean plasma concentration-time profiles of amoxicillin are shown in Fig. 2. As observed in Fig. 2 and Table 2, Ro 64-0802 had little effect upon the pharmacokinetics of amoxicillin as the plasma and urine parameters in the presence and absence of oseltamivir were comparable. The ratio of means and corresponding 90% confidence interval estimates for C_{max} and AUC_{0-12} of amoxicillin in the presence versus in the absence of steady-state concentrations of the active metabolite Ro 64-0802 were within the 80 to 125% bioequivalence range. Similarly, as observed in Fig. 3 and Table 3, amoxicillin had little effect upon Ro 64-0802 pharmacokinetics. The ratio of means and corresponding 90% confidence interval estimates for C_{max} and AUC_{0-12} of Ro 64-0802 in the presence versus the absence of single-dose concentrations of amoxicillin were within the 80 to 125% bioequivalence range.

Inhibition of hOAT1-Mediated Transport of p-Aminohippuric Acid. Under steady-state kinetic conditions, the uptake of PAH into CHO^{hOAT} cells was measured in the presence of various concentrations of the competitor drugs, and inhibition constants (K_i) were determined from Lineweaver-Burke plots (Fig. 4). The mean K_i

TABLE 1

Summary of mean (S.D.) pharmacokinetic parameters, ratios, and 90% confidence intervals for the active metabolite Ro 64-0802 following a single oral dose of 150 mg of oseltamivir given alone, with cimetidine (400 mg q.i.d.), or with probenecid (500 mg q.i.d.) in healthy volunteers ($n = 18$)

Ro 64-0802 Parameter	Regimen A (alone) Mean (S.D.)	Regimen B (with Cimetidine)		Regimen C (with Probenecid)	
		Mean (S.D.)	Ratio B/A (90% CI)	Mean (S.D.)	Ratio C/A (90% CI)
			%		%
C_{max} (ng/mL)	587 (136)	599 (198)	102 (88, 116) ^a	1093 (325)	186 (173, 200)
AUC_{∞} (ng · h/mL)	6597 (1225)	6992 (1850)	106 (83, 129)	16603 (5091)	252 (229, 275)
t_{max} (h)	4.5 (1.1)	4.1 (0.8)	N.A. ^b	5.3 (1.1)	N.A.
fe (%)	71.5 (16.7)	69.6 (10.9)	N.A.	78.0 (25.8) ^c	N.A.
CL_R (l/h)	15.7 (4.4)	15.0 (4.4)	N.A.	7.5 (3.9) ^c	N.A.

CI, confidence interval.

^a Ratio of means (log transformed) of the 90% CI were within the 80 to 125% bioequivalence range.

^b Not applicable.

^c $n = 17$.

TABLE 2

Summary of mean (S.D.) values, ratios, and 90% confidence intervals of pharmacokinetic parameters of amoxicillin (500 mg) in the absence (Regimen A) and presence (Regimen C) of oseltamivir (75 mg b.i.d.) in healthy volunteers ($n = 12$)

Amoxicillin Parameter	Regimen A Mean (S.D.)	Regimen C Mean (S.D.)	Ratio C/A (90% CI)
			%
C_{max} (μ g/mL)	5.5 (2.0)	5.5 (1.8)	100 (85, 116) ^a
AUC_{0-12} (μ g · hr/mL)	16.1 (3.6)	15.7 (4.5)	97.5 (89, 107) ^a
t_{max} (h)	2.0 (0.9)	2.0 (0.6)	N.A. ^b
fe (%)	49.1 (11.5)	53.6 (16.1)	N.A.
CL_R (l/h)	15.5 (4.5)	17.5 (5.4)	N.A.

CI, confidence interval.

^a Ratio of means (log transformed) of the 90% CI were within the 80 to 125% bioequivalence range.

^b Not applicable.

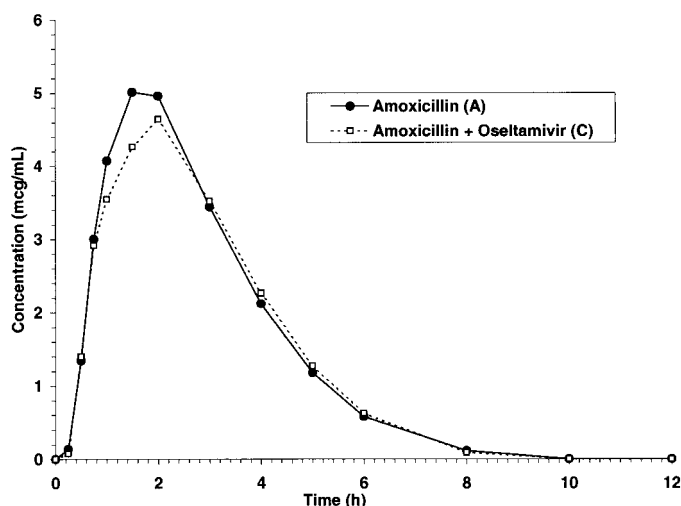


FIG. 2. Mean plasma concentrations of amoxicillin (500 mg) in the absence and presence of oseltamivir (75 mg b.i.d.) in healthy volunteers ($n = 12$).

values for the three tested drugs were as follows: probenecid 0.0043 mM ($n = 2$), amoxicillin 7.55 mM ($n = 2$), and Ro 64-0802 45.1 mM ($n = 3$). Consistent with previously published data (Ho et al., 2000), probenecid exhibited a strong inhibitory effect on the PAH transport mediated by hOAT1. In contrast, amoxicillin and Ro 64-0802 were very weak inhibitors, with K_i values approximately 1,800- and 10,000-fold higher, respectively, than probenecid.

Effect of Ro 64-0802 and Probenecid on hOAT1-Mediated Transport of Amoxicillin. The effects of Ro 64-0802 and probenecid

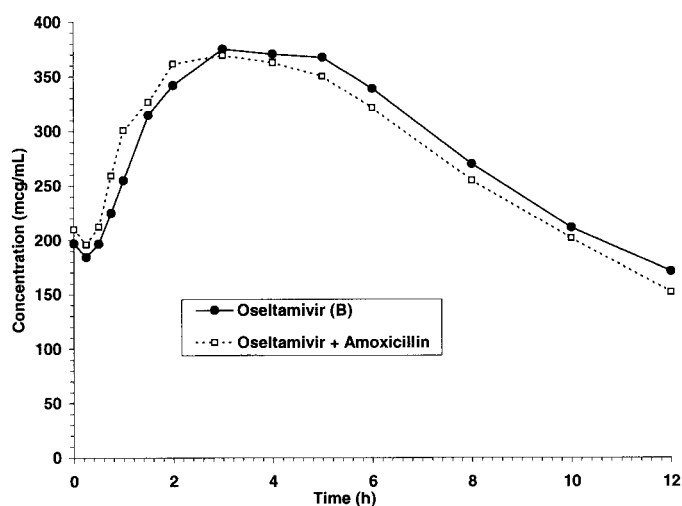


FIG. 3. Mean steady-state (75 mg of oseltamivir b.i.d.) plasma concentrations of Ro 64-0802 in the absence and presence of amoxicillin (500 mg) in healthy volunteers ($n = 12$).

TABLE 3

Summary of mean (S.D.) values, ratios, and 90% confidence intervals of steady-state (oseltamivir 75 mg b.i.d.) pharmacokinetic parameters of Ro 64-0802 (active metabolite) in the absence (Regimen B) and presence (Regimen C) of amoxicillin (500 mg) in healthy volunteers ($n = 12$)

Ro 64-0802 Parameter	Regimen B Mean (S.D.)	Regimen C Mean (S.D.)	Ratio C/B (90% CI)
			%
C_{max} (ng/mL)	389 (59)	397 (74)	102 (98, 106) ^a
AUC_{0-12} (ng · hr/mL)	3438 (439)	3330 (425)	96.8 (95, 99) ^a
t_{max} (h)	3.7 (1.1)	3.3 (1.3)	N.A. ^b
fe (%)	83.1 (6.2)	84.9 (6.2)	N.A.
CL_R (l/h)	16.8 (2.5)	17.7 (2.5)	N.A.

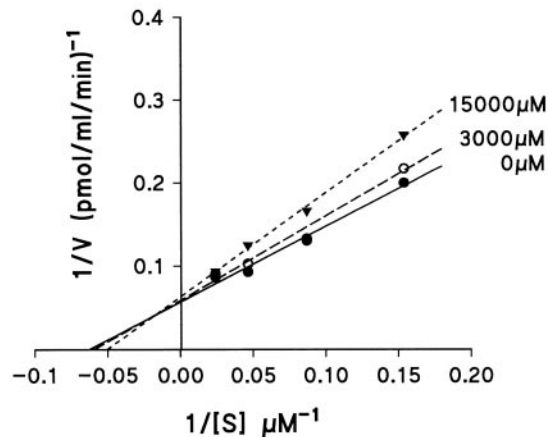
CI, confidence interval.

^a Ratio of means (log transformed) of the 90% CI were within the 80 to 125% bioequivalence range.

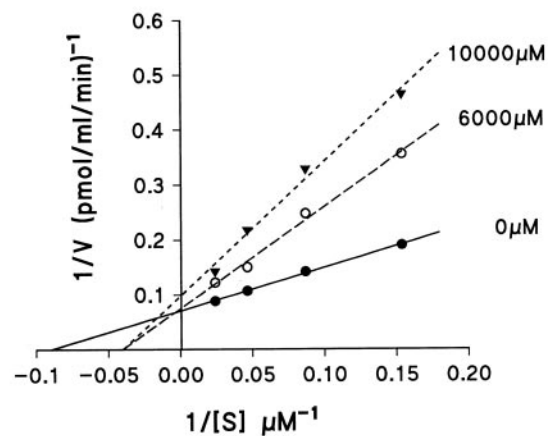
^b Not applicable.

on the uptake of amoxicillin via hOAT1 are summarized in Table 4. Cells expressing functional hOAT1 accumulated 10 μ M [³H]amoxicillin approximately 2-fold more efficiently than the inactive control cells. In the presence of 500 μ M probenecid, the uptake of amoxicillin was similar in both cell lines, indicating that amoxicillin is a substrate for hOAT1, although the uptake proceeds with a low efficiency. However, unlike 500 μ M probenecid, Ro 64-0802 at the same concentration did not interfere with the hOAT1-specific uptake of amoxicillin into CHO^{hOAT} cells (Table 4).

A) Ro 64-0802



B) Amoxicillin



C) Probenecid

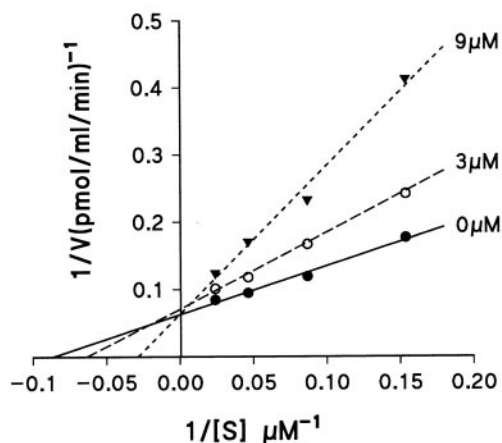


FIG. 4. Lineweaver-Burke plots showing the inhibition of the hOAT1-mediated transport of *p*-aminohippuric acid (5–40 μM) in CHO^{hOAT} cells for Ro 64-0802 (A), amoxicillin (B), and probenecid (C) (mean values of $n = 2$ experiments for amoxicillin and probenecid and $n = 3$ for Ro 64-0802).

Effect of Amoxicillin and Probenecid on hOAT1-Mediated Transport of Ro 64-0802. As shown in Table 4, the intracellular accumulation of 250 μM [^{14}C]Ro 64-0802 was approximately 2-fold higher in the hOAT1-positive cells than that in control cells lacking

the functional renal transporter, demonstrating that, similar to amoxicillin, Ro 64-0802 is a weak substrate for hOAT1. In the presence of 500 μM probenecid, the accumulation of Ro 64-0802 in CHO^{hOAT} cells was similar to that in the control cells. In contrast, 500 μM amoxicillin showed no effect on the uptake of Ro 64-0802 in either cell line, indicating that unlike probenecid, amoxicillin does not interfere with the transport of Ro 64-0802 mediated by hOAT1. Preloading of CHO^{hOAT} cells with 5 mM glutarate increased the uptake of Ro 64-0802 by approximately 25% compared with preincubation in a buffer lacking glutarate (4.75 ± 0.57 versus 3.77 ± 0.55 pmol/ 10^6 ; $n = 3$). This effect of glutarate preloading was not observed in control $\text{CHO}^{\text{PIRES}}$ cells (2.09 ± 0.19 versus 2.08 ± 0.63 pmol/ 10^6 ; $n = 3$), indicating further that Ro 64-0802 is recognized by hOAT1 as a substrate.

Discussion

The pharmacokinetic parameters of the active metabolite Ro 64-0802, rather than the parameters of the prodrug oseltamivir, is used in the evaluation of renal drug-drug interaction. This is because oseltamivir is rapidly and extensively hydrolyzed to Ro 64-0802, which is the predominant moiety in plasma and urine.

Study 1 was undertaken to identify the active pathway by which Ro 64-0802 is secreted renally. In the presence of steady-state concentrations of probenecid, a potent competitive inhibitor of the renal tubular secretion of weak organic acids, an altered pharmacokinetic profile was observed for the oseltamivir active metabolite Ro 64-0802. The major change was a greater than 50% decrease in the renal clearance, which resulted in a 1.9-fold increase in C_{max} and 2.5-fold increase in AUC_{∞} . This indicates that the renal tubular secretion of Ro 64-0802 occurs via the anionic transport process, a pathway known to be involved in the elimination of acidic drugs. Coadministration of probenecid reduced the renal clearance of Ro 64-0802 from 15.7 l/h (262 ml/min), a value exceeding the glomerular filtration rate, to 7.5 l/h (125 ml/min), a value equal to glomerular filtration rate, suggesting complete inhibition of tubular secretion of Ro 64-0802 (Ro 64-0802 is not protein bound). Although Ro 64-0802 is actively secreted, its renal clearance is only about 39% of renal plasma flow rate (674 ml/min). This is in contrast to PAH, a substrate known to be avidly secreted via the renal tubules (renal clearance = 600 ml/min), and it is almost completely removed (90%) from the blood in a single pass through the kidney (Ritschel, 1992). This suggests that Ro 64-0802 may be a weak competitor for the anionic tubular secretion pathway and that its ability to compete effectively with other drugs secreted via the anionic transport process may be limited. The results of this study also indicate that drugs that reduce renal tubular secretion by the anionic transport process are likely to inhibit Ro 64-0802 elimination. However, even under conditions designed to severely reduce elimination by this pathway, only a 2.5-fold increase in total systemic exposure to Ro 64-0802 occurred. This is not considered to be clinically relevant compared with the large safety margin shown in this and previous clinical studies with oseltamivir (He et al., 1999b; Oo et al., 2001).

Study 1 also demonstrated that the renal clearance and plasma pharmacokinetic parameters of Ro 64-0802 were not appreciably altered when given in the presence of steady-state plasma concentrations of cimetidine, a known competitor for renal tubular secretion of predominantly basic or cationic drugs. This indicates that secretion of Ro 64-0802 does not involve the cationic secretion transport process. Therefore, there is unlikely to be any competitive drug interaction with other drugs excreted via this pathway. This study, therefore, provides supportive evidence that the presence of cimetidine or other drugs that inhibit hepatic enzymes will not alter the pharmacokinetics

TABLE 4

Uptake of amoxicillin and Ro 64-0802 into CHO^{hOAT} and control CHO^{PIRES} cells in the absence or presence of inhibitors

Mean (S.D.) from four independent experiments; *, $P < 0.02$; **, $P < 0.005$ using a paired two sample t test.

Inhibitor ^a	Uptake			
	Amoxicillin (10 μ M)		Ro 64-0802 (250 μ M)	
	CHO ^{hOAT} Cells	CHO ^{PIRES} Cells	CHO ^{hOAT} Cells	CHO ^{PIRES} Cells
	<i>pmol/10⁶ cells/30 min</i>			
None	3.38 (0.84)*	1.41 (0.29)*	3.86 (0.90)**	2.16 (0.48)**
Probenecid	1.82 (0.46)	1.84 (0.34)	2.01 (0.65)	1.81 (0.44)
Amoxicillin	N.A. ^b	N.A.	4.22 (0.94)*	2.30 (0.38)*
Ro 64-0802	3.19 (0.78)*	1.39 (0.31)*	N.A.	N.A.

^a Each inhibitor tested at 500 μ M concentration.

^b Not applicable.

of Ro 64-0802, as cimetidine is a known nonspecific inhibitor of the cytochrome P450 isoenzymes.

Study 2 was undertaken to investigate whether oseltamivir could affect the pharmacokinetics of other drugs that are excreted via the anionic pathway of renal tubular secretion. Amoxicillin was selected as a suitable drug to be coadministered since it is primarily eliminated by this pathway and is a zwitterion as for Ro 64-0802. In this study, multiple doses of Ro 64-0796 were administered to assess interaction with amoxicillin in the presence of a steady-state concentration of Ro 64-0802. Due to the short elimination half-life (1–1.5 h) of amoxicillin, there will be no drug accumulation even if amoxicillin is administered t.i.d.; hence, a single dose of amoxicillin was used. The study results demonstrated that the renal clearance of Ro 64-0802 was not inhibited and the primary plasma pharmacokinetic parameters of amoxicillin and Ro 64-0802 (AUC and C_{max}) were in fact equivalent in the absence or presence of concomitant drug administration. These results further confirmed that Ro 64-0802 is a weak competitor for the anionic renal tubular secretion processes, and its potential to inhibit other renal secreted organic acids is minimal.

In vitro experiments were also carried out to address the interactions of Ro 64-0802 with hOAT1, a recently identified human renal transporter that plays the main role in the active tubular secretion of organic acids. In CHO^{hOAT} cells expressing functional hOAT1, Ro 64-0802 showed only a marginal effect on the transport activity of hOAT1, as demonstrated by its very high K_i value (45.1 mM) for the inhibition of hOAT1-specific transport of PAH. Also, in contrast to the potent hOAT1 inhibitor probenecid, 500 μ M Ro 64-0802 did not show any effect on the hOAT1-mediated transport of amoxicillin, another organic acid undergoing renal tubular secretion. The concentration of Ro 64-0802 used in this study exceeds its maximum therapeutic plasma concentration ($C_{max} = 389$ ng/ml; 1.3 μ M) by almost 400-fold (He et al., 1999a).

In addition, the in vitro studies demonstrated that hOAT1 was able to transport Ro 64-0802. The process, however, was found to proceed with a considerably lower efficiency compared with the prototype organic anion substrate PAH. After a 30-min incubation, the net hOAT1-mediated uptake of Ro 64-0802 (250 μ M) into CHO^{hOAT} cells accounted for approximately 1.9 pmol/10⁶ cells (Table 4). Under the same experimental conditions, PAH at a significantly lower concentration (5 μ M) was found to be a much more efficient substrate for hOAT1, with a net hOAT1-mediated transport of 27 pmol/10⁶ cells. An attempt was made to determine the kinetics of the hOAT1-mediated transport of Ro 64-0802. However, due to the low affinity and limited transport efficiency, large quantities of radioactive Ro 64-0802 had to be used, producing a considerably high background and unacceptable variations in the detected transport. This did not allow for an accurate determination of the transport kinetic constants.

Despite the low in vitro efficiency of hOAT1-mediated transport of Ro 64-0802, hOAT1 may still play a key role in renal excretion of this metabolite. First, the apparent inefficiency of hOAT1-mediated transport of Ro 64-0802 is derived from the rate of its uptake into CHO^{hOAT} cells, which depends on the level of hOAT1 expression. The expression of hOAT1 is most probably substantially lower in our in vitro system compared with renal proximal tubules. Second, because hOAT1 functions as organic anion/dicarboxylate exchanger, its transport activity depends on intracellular concentration of dicarboxylic acids, predominantly α -ketoglutarate. Because of the presence of basolateral (SDCT2) and luminal (NaDC-1) dicarboxylate transporters in proximal tubules (Sekine et al., 1998; Chen et al., 1999), the intracellular concentration of dicarboxylic acids is likely to be substantially higher in proximal tubules than in CHO^{hOAT} cells, resulting in more efficient in vivo hOAT1-mediated transport.

In addition to hOAT1, other organic anion transporters, such as hOAT3 (Cha et al., 2001; Takeda et al., 2001), hOAT4 (Cha et al., 2000), and OATP (Kullak-Ublick et al., 1995) have been identified in human kidney, which may potentially mediate active renal excretion of Ro 64-0802. Among them, only hOAT3 has been precisely localized to the basolateral membrane of proximal tubules and, thus, is expected to participate in active excretion of organic anions from blood into tubular lumen (Cha et al., 2001). hOAT3, however, is sensitive to cimetidine (Cha et al., 2001), and our pharmacokinetic studies demonstrated that cimetidine does not reduce renal excretion of Ro 64-0802. Precise renal localization and function of hOAT4 and OATP has yet to be determined. In addition, OATP has been shown to be insensitive to probenecid (Kullak-Ublick et al., 1995), an efficient inhibitor of renal excretion of Ro 64-0802. Oatp1, a rat transporter closely related to OATP, is expressed in the luminal (apical) membrane of proximal tubules (Bergwerk et al., 1996), suggesting that this transporter might be involved in reabsorption of organic anions from glomerular filtrate rather than in active excretion of anions from blood circulation. Nevertheless, because of the emerging complexity of renal transport of small organic molecules, interactions of Ro 64-0802 with newly identified renal transporters warrant further studies.

In conclusion, the lack of the interference of Ro 64-0802 with the hOAT1-mediated transport of amoxicillin and vice versa observed in the in vitro cell-based hOAT1 assay and the low affinity of Ro 64-0802 for this transporter were consistent with the findings from the in vivo pharmacokinetic drug interaction studies. Hence, this novel in vitro model could provide a useful screening system for evaluating drug-drug interaction via the hOAT1-mediated renal tubular transport. The study results also demonstrate that Ro 64-0802 is secreted by the anionic renal tubular transporter, and oseltamivir has a low drug-drug interaction potential at the renal tubular level.

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