

RESEARCH ARTICLE

Therapeutic Drug Monitoring in Interstitial Fluid: A Feasibility Study Using a Comprehensive Panel of Drugs

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Received 13 May 2012; revised 23 July 2012; accepted 10 August 2012

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23309

ABSTRACT: This study compared drug concentration–time profiles in interstitial fluid (ISF) and blood, using an established animal model and a comprehensive panel of drugs, to examine the feasibility of therapeutic drug monitoring (TDM) in ISF. An intravenous bolus of vancomycin, gentamicin, tacrolimus, cyclosporine, mycophenolate, valproic acid, phenobarbital, phenytoin, carboplatin, cisplatin, methotrexate, theophylline, or digoxin was administered into the ear vein ($n = 4–6$) of rabbits. Serial (0–72 h after dose) blood and ISF concentrations (collected via an ultrafiltration probe) were determined by validated analytical assays. Pharmacokinetic parameters were generated by noncompartmental analysis. Vancomycin, gentamicin, and carboplatin showed no significant difference in area under the curve (AUC) values in ISF and blood, respectively. Other AUCs were lower (mycophenolic acid, valproic acid, phenobarbital, cisplatin, methotrexate, theophylline, and digoxin) or not measurable (tacrolimus, cyclosporine, and phenytoin) in ISF with our extraction technique. Similar concentration–time profiles in the two matrices were evident for a selection of drugs tested. Using a comprehensive panel of drugs in a single experimental setting, we have identified agents that can be quantified in ISF. Our newly developed scoring algorithm can help determine the feasibility of conducting TDM in ISF. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association *J Pharm Sci*

Keywords: therapeutic drug monitoring; pharmacokinetics; disposition; distribution; clinical pharmacokinetics; interstitial fluid; rabbit model; ultrafiltration probe; vancomycin; methotrexate

INTRODUCTION

Therapeutic drug monitoring (TDM) involves the quantification of drugs for the purpose of improving efficacy or minimizing toxicity in patient care. In general, TDM is performed for drugs with a narrow therapeutic index where clear concentration–response relationships exist. On the basis of a previously published clinical decision-making algorithm,¹

various therapeutic agents commonly used today are deemed appropriate for TDM.

Therapeutic drug monitoring typically involves the determination of trough concentration (C_{\min}), peak concentration (C_{\max}), or exposure to the drug [area under the curve (AUC)], each of which usually necessitates the sampling of blood. Although it is usually not a problem obtaining blood from most populations, there are groups of patients (e.g., those with fragile veins, neonates, infants, and children) for whom frequent blood drawing may prove difficult or impossible. Body fluids, which are accessible through a less invasive technique (e.g., tear fluid, saliva, urine, and feces) can also be used for drug concentration determination,² but are suitable only in specific circumstances where a sufficient amount of drug is present. The lack of consistency or reliability

Abbreviations used: AUC, area under the curve; C_{\max} , peak concentration; C_{\min} , trough concentration; ISF, interstitial fluid; PK, pharmacokinetics; TDM, therapeutic drug monitoring; T_{\max} , time-to-peak concentration.

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Journal of Pharmaceutical Sciences

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with respect to drug measurement and insufficient knowledge of concentration–response relationships are the challenges when using nonblood matrices for TDM.

An ideal matrix for TDM would resemble blood but could be sampled in a minimally invasive manner. In this context, we are proposing to use interstitial fluid (ISF), which has a composition close to plasma without the plasma proteins, as an alternative. ISF consists of an aqueous solution of amino acids, carbohydrates, fatty acids, drugs, and other micronutrients capable of passing through capillary walls. The sampling technique can be minimally invasive and potentially pain free. Because ISF contains minimal quantities of cells and proteins, it requires little preparation for drug analysis. However, the use of ISF for TDM can be complicated by drug transport processes into/out of that matrix that may result in altered pharmacokinetic (PK) parameters compared with the blood [e.g., glucose presents in ISF with a delayed time-to-peak concentration (T_{\max})].^{3,4} Site-specific differences in ISF drug concentration also exist (e.g., vancomycin levels obtained in the abdominal wall differ from those in brain tumor⁵), which means consistency in drug sampling is crucial.

The best proof-of-concept example for using ISF in TDM is the subcutaneous glucose sensor for the insulin-dependent diabetic population.⁴ Although investigations on ISF PK have been published for multiple molecules, most of these studies have focused on demonstrating adequate antibiotic penetration into the ISF to calculate ratios of drug level per minimal inhibitory concentration.^{6–7} As such, systematic investigations using drugs belonging to a wide variety of therapeutic classes remain to be carried out to test the general hypothesis that ISF can be a viable equivalent to blood as a suitable alternative matrix for TDM.

The objective of the current study was to demonstrate the feasibility of sampling and obtaining reliable drug concentrations in ISF by comparing PK parameters (i.e., C_{\max} , T_{\max} , and AUC) obtained in ISF and blood. Using an established animal model,⁸ an extensive list of contemporary drugs that are used in clinical TDM and that represent a wide variety of therapeutic classes was used: antibiotics (vancomycin and gentamicin), immunosuppressants (tacrolimus, cyclosporine, and mycophenolate mofetil), anticonvulsants (valproic acid, phenobarbital, and phenytoin), chemotherapeutic drugs (carboplatin, cisplatin, and methotrexate), and miscellaneous agents (theophylline and digoxin). To our knowledge, this is the first instance where such a wide selection of drugs has been tested in a single experimental setting.

MATERIALS AND METHODS

The current study was approved by the Animal Care Committee at The University of British Columbia and adhered to the Principles of Laboratory Animal Care (NIH publication #85-23, revised in 1985). New Zealand White rabbits (1.75–5.5 kg) obtained from Charles River Laboratories (Wilmington, Massachusetts) were used for the study. Rabbits were acclimatized for at least 7 days in group pens in a temperature-controlled room under a 12 h dark/light cycle and fed typical diet and water *ad libitum* before and after testing procedures.

Drugs that warrant TDM in the clinic were used in this study. Vancomycin [vancomycin hydrochloride United States Pharmacopeia (USP) 500 mg; Hospira, Lake Forest, Illinois], gentamicin (gentamicin injection USP 40 mg/mL; Sandoz, Boucherville, Quebec, Canada), tacrolimus (Prograf[®] injection 5 mg/mL; Astellas Pharma, Markham, Ontario, Canada), cyclosporine (Sandimmune[®] injection 50 mg/mL; Novartis, Dorval, Quebec, Canada), mycophenolate mofetil [CellCept[®] intravenous (i.v.) 500 mg; Roche, Mississauga, Ontario, Canada], valproic acid (sodium valproate 50 mg/kg; Sigma, St. Louis, Missouri), phenobarbital (sodium phenobarbital 30 mg/kg; Sigma), phenytoin sodium (phenytoin sodium injection USP 50 mg/mL; Sandoz), carboplatin (carboplatin injection 10 mg/mL; Hospira), cisplatin (cisplatin injection 1 mg/mL; Hospira), methotrexate (methotrexate injection 25 mg/mL; Mayne Pharma, Salisbury South, South Australia, Australia), theophylline (theophylline 12 mg/kg; Sigma), and digoxin (digoxin injection 0.25 mg/mL; Sandoz). Tacrolimus, valproic acid, theophylline, and phenobarbital were diluted in sterile NaCl 0.9% and sterile filtered. The ultrafiltration (UF) probe (reinforced UF-3-12) was obtained from Bioanalytical Systems Inc. (West Lafayette, Indiana).

Rabbits were premedicated with 22.5 mg/kg ketamine (Ketaset[®]; Wyeth, Guelph, Ontario, Canada) and 2.5 mg/kg xylazine (Rompun[®], Bayer, Toronto, Ontario, Canada) before the implantation of the UF probe. Rabbits were maintained on oxygen and isoflurane (AERRANE[®], Baxter Corporation, Mississauga, Ontario, Canada) if needed. The implantation site was infiltrated with 0.3 mL bupivacaine (Marcaine[®] 0.50%; Hospira) subcutaneously. Heart rate, respiration rate, body temperature, and oxygen saturation of hemoglobin (pulse oximetry) were monitored during the procedure. To insert the UF probe, the fur around the implant site was shaved and the skin aseptically prepped. The UF probe (7 cm) was placed subcutaneously between the shoulders with a trocar, and the tubing of the probe held in place with butterfly tape

tabs that were sutured to the rabbit skin. After the procedure, the implant site was covered with sterile Bioclusive® adhesive dressing (Systagenix, Gargrave, North Yorkshire, UK) and rabbits were fitted with a special jacket (Lomir Biomedical Inc., Malone, New York) to prevent any unintended removal of the probe due to self-scratching. After 1 day of recovery, slow i.v. injections (over 1–2 min) of nontoxic doses of drugs were administered to rabbits via the ear vein (see legend to Fig. 1 for the specific doses used). The other ear was reserved for the collection of blood via an arterial over-the-needle catheter. Blood samples were collected at the following time points: predose; 15, 30, 45, and 60 min; 2, 4, 6, 8, and 24 h (and the additional 72 h time point for phenobarbital), whereas ISF was collected (via the UF probe) over time in-

tervals: predose; 0–15, 15–30, 30–45, and 45–60 min; 1–2, 2–4, 4–6, 6–8, and 23–24 h (71–72 h for phenobarbital). Graphs (Fig. 1) were shown as either 8, 24, or 72 h plots and only captured all concentrations above limits of quantitation (i.e., if the 24 h concentration was below the limit of quantitation, then only the 8 h plot was generated). Only free drug was measured in ISF as the UF probe had a pore size (30 kDa) that limited the passage of protein-bound drugs from blood.

Drug samples were analyzed at Exova (Surrey, British Columbia, Canada) and in clinical laboratories at Vancouver General Hospital and Children's and Women's Hospital (Vancouver, British Columbia, Canada) using validated assays. Methotrexate and valproic acid were analyzed in ethylenediaminetetraacetic acid (EDTA)-plasma using fluorescence

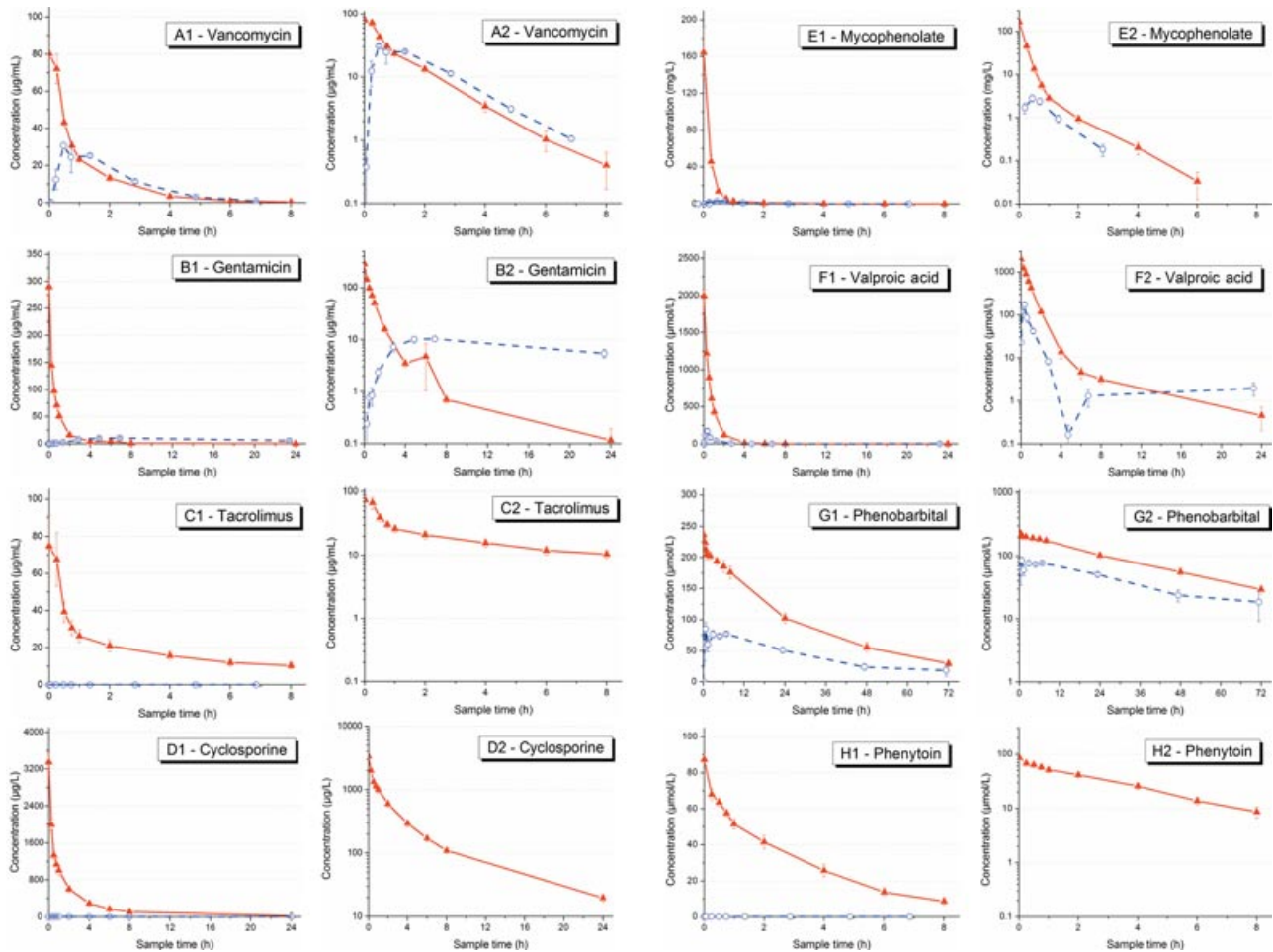


Figure 1. Concentration–time profiles in (○) ISF and (▲) blood in New Zealand White rabbits. Data are expressed as mean \pm SEM ($n = 4\text{--}6$ rabbits). See *Materials and Methods* section for details on dosing, sample collection regimens, and drug analysis methods. (A1 and A2) Vancomycin (20 mg/kg), (B1 and B2) gentamicin (50 mg/kg), (C1 and C2) tacrolimus (0.1 mg/kg), (D1 and D2) cyclosporine (5 mg/kg), (E1 and E2) mycophenolate (40 mg/kg), (F1 and F2) valproic acid (50 mg/kg), (G1 and G2) phenobarbital (30 mg/kg), (H1 and H2) phenytoin (10 mg/kg), (I1 and I2) carboplatin (18.7 mg/kg), (J1 and J2) cisplatin (3 mg/kg), (K1 and K2) methotrexate (15 mg/kg), (L1 and L2) theophylline (12 mg/kg), and (M1 and M2) digoxin (0.02 mg/kg). C_{\max} and AUC values are presented in Table 1(Continued).

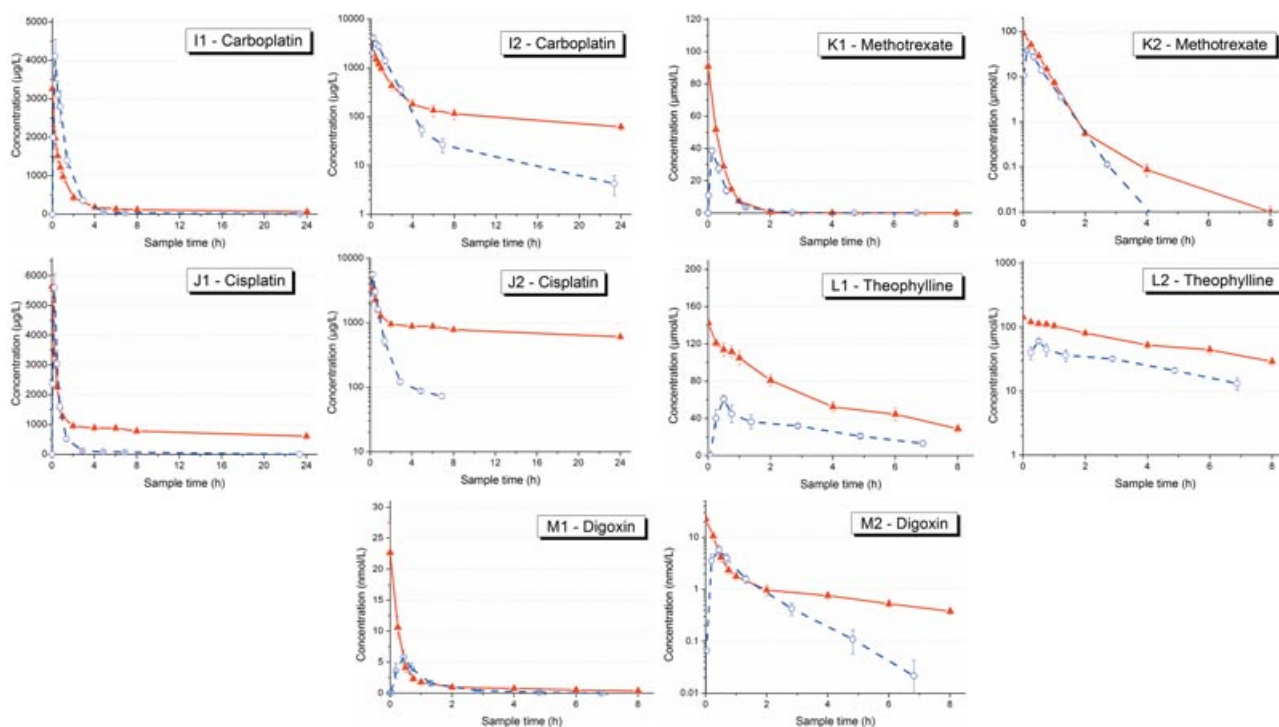


Figure 1. Continued.

polarization immunoassays (Abbott Laboratories, Abbott Park, Illinois). Particle-enhanced turbidimetric inhibition immunoassays (Siemens, Deerfield, Illinois) were used to analyze phenytoin, digoxin, phenobarbital, vancomycin, and gentamicin in serum. Mycophenolic acid (active moiety of mycophenolate mofetil), tacrolimus, and cyclosporine were analyzed in EDTA-whole blood using liquid chromatography–tandem mass spectrometry. A platinum trace analysis with inductively coupled atomic emission spectrometry (United States Environmental Protection Agency, 6010 C) for carboplatin and cisplatin in EDTA-whole blood was conducted at Exova. For all analytical assays, ISF samples were not subjected to sample processing, and all samples were stored at -20°C until analysis.

Drug recovery from the UF probe (UF-3-12) was performed to account for possible interactions between the drugs and the probe materials (membrane: polyacrylonitrile, tubing: fluorinated ethylene propylene). All 13 drugs have significantly smaller molecular weights (0.18–1.45 kDa) than the molecular weight cutoff (MWCO) of the UF probes (30 kDa). Briefly, the UF probes were soaked overnight in distilled water and washed with phosphate-buffered saline (PBS) pH 7.4, the next day. After connecting the probe to a 21 G \times 3/4" winged infusion set, it was immersed in a drug solution of known concentration at room temperature. To mimic the experimental conditions *in vivo*, drug concentrations for the recovery experiment were chosen close to the maximum con-

centration detected in blood or ISF. The first 150 μL of extract was discarded to completely purge the remaining PBS in the probe. Three consecutive extracts of 150 μL each were then collected in vacuum tubes and quantified using the aforementioned analytical assays. To calculate drug recovery, drug concentrations in the three extracts were compared with the initial drug solution (also quantified with the same analytical techniques) and expressed as percentage of recovery [recovery (%) = concentration (after passing UF probe)/concentration (before passing UF probe) \times 100].

Peak concentration, T_{max} , and AUC were determined by noncompartmental PK analysis (WinNonlin 5.1; Pharsight Corporation, Cary, North Carolina). The zero-time concentrations of the blood data were extrapolated using rate constants obtained from linear portions of the concentration–time curves (specific to each drug). The mid-point of the ISF collection interval was used for data plotting and analysis after subtracting the time required for each drug to traverse the UF probe. For this purpose, the overall length of probe and collection needle for each rabbit (e.g., 80 mm) was divided by the individual ISF flow (e.g., 3.2 $\mu\text{L}/\text{min}$), which was determined in each rabbit during the first hour of ISF collection. This was further divided by the calibrated and constant length of tubing, which contained 1 μL of the solvent (3.2 mm), yielding probe traversing times of between 4 and 9 min. Data were expressed as mean \pm SD in text and tables or mean \pm SEM in figures (units of

Table 1. Comparison of C_{\max} and AUC Values Between ISF and Blood

	C_{\max}		AUC	
	ISF	Serum/Blood	ISF	Serum/Blood
Antibiotics				
Vancomycin ^a	32.1 ± 2.6*	80.2 ± 18.5	75.3 ± 7.7	89.8 ± 15.7
Gentamicin	10.8 ± 3.6*	289.5 ± 40.3	176.1 ± 54.0	188.8 ± 44.7
Immunosuppressants				
Tacrolimus	ND	74.7 ± 32.8	ND	157.3 ± 48.7
Cyclosporine	ND	3349.2 ± 536.9	ND	5144.4 ± 1129.5
Mycophenolic acid	2.6 ± 1.3*	164.2 ± 69.3	3.2 ± 1.3*	40.3 ± 15.9
Anticonvulsants				
Valproic acid	178.2 ± 56.6*	1992.5 ± 217.6	177.5 ± 32.5*	1424.9 ± 213.3
Phenobarbital	95.6 ± 16.8*	239.3 ± 20.1	2822.4 ± 274.5*	6700.4 ± 1199.2
Phenytoin	ND	87.4 ± 8.7	ND	241.4 ± 49.3
Chemotherapy				
Carboplatin	4110.5 ± * 1058.9	3259.3 ± 730.1	5667.3 ± 1755.6	5004.1 ± 2049.9
Cisplatin	5600.0 ± 1017.1	5639.0 ± 799.9	4002.3 ± 826.7*	20128.1 ± 1328.1
Methotrexate	36.3 ± 8.7*	90.8 ± 10.9	19.7 ± 5.2*	39.4 ± 5.2
Miscellaneous				
Theophylline	62.6 ± 7.1*	142.5 ± 12.4	190.8 ± 51.8*	513.9 ± 103.7
Digoxin	5.9 ± 2.9*	22.7 ± 13.2	6.4 ± 3.2*	14.6 ± 6.9

Data are presented as mean ± SD.

C_{\max} , maximum concentration; AUC, area under the concentration–time curve; ND, not detectable–below detection limit; ISF, interstitial fluid.

Concentration units: vancomycin (μg/mL), gentamicin (μg/mL), tacrolimus (μg/mL), cyclosporine (μg/L), mycophenolic acid (mg/L), valproic acid (μmol/L), phenobarbital (μmol/L), phenytoin (μmol/L), carboplatin (μg/L), cisplatin (μg/L), methotrexate (μmol/L), theophylline (μmol/L), and digoxin (nmol/L). The time unit for calculating AUC was “hour”. (Units of measure named above are those commonly used in the clinic and reported by clinical laboratories.)

* $p < 0.05$ ($n = 6$, except for vancomycin and gentamicin, where $n = 4$).

^aFrom pilot study⁸.

measure were those commonly used in the clinic and reported by the clinical laboratories named above), and differences between groups were assessed by paired Student's t -test. Concentration–time profiles of drugs in ISF were considered “similar” to blood if the calculated terminal elimination rate constants in both matrices were within 25%. The level of significance was set, a priori, at a α level of 0.05.

RESULTS

Antibiotics: Concentration–Time Profiles in ISF and Blood

Vancomycin, a glycopeptide, is the mainstay of treatment for Gram-positive methicillin-resistant *Staphylococcus aureus* infections.⁹ A nontoxic dose of vancomycin (20 mg/kg) was administered in this study. In our model ($n = 4$), vancomycin exhibited reduced C_{\max} in ISF (32.1 ± 2.6 μg/mL, mean ± SD) compared with the blood (80.2 ± 18.5 μg/mL) ($p < 0.05$), but had similar AUCs in the two matrices (75.3 ± 7.7 vs. 89.8 ± 15.7 μg h/mL), respectively (Table 1). A delayed average T_{\max} of 0.66 h was observed in ISF. The concentration–time profiles for vancomycin in both matrices were similar (Figs. 1-A1 and 1-A2, Table 1), and this is evident by the parallel decline in their terminal elimination phases in log-transformed plots (Fig. 1-A2, Table 2).

Gentamicin, an aminoglycoside, is widely used to treat a variety of Gram-negative infections.¹⁰ The dose of gentamicin (50 mg/kg) in our rabbit study ($n = 6$) was based on the published data on rabbits.¹¹ Similar to vancomycin, gentamicin showed a reduced C_{\max} in ISF (10.8 ± 3.6 μg/mL, mean ± SD) compared with the blood (289.5 ± 40.3 μg/mL) ($p < 0.05$) and similar AUCs (176.1 ± 54.0 vs. 188.8 ± 44.7 μg h/mL, respectively) (Table 1). In contrast to vancomycin, the delay in T_{\max} for gentamicin was substantial (average of 6.2 h), and this, together with the markedly reduced C_{\max} , generated a concentration–time profile in ISF that was dissimilar to that in blood (Figs. 1-B1 and 1-B2, Table 2).

Immunosuppressant Drugs: Concentration–Time Profiles in ISF and Blood

The calcineurin inhibitors tacrolimus and cyclosporine and the antimetabolite mycophenolate are the mainstay antirejection drugs for various types of solid organ and bone marrow transplants.¹² Nontoxic doses (based on the published data on rabbits) of tacrolimus (0.1 mg/kg), cyclosporine (5 mg/kg), and mycophenolate mofetil (40 mg/kg) were used in this study.^{13,14} Despite readily detectable levels in blood, tacrolimus ($n = 4$) and cyclosporine ($n = 6$) were not measurable in ISF (Figs. 1-C and 1-D, Table 1). Only a limited number of mycophenolate mofetil ($n = 6$) samples (which is hydrolyzed to mycophenolic acid)

Table 2. Comparison of Terminal Elimination Constant (k) and half-life ($t_{1/2}$) Values Between ISF and Blood

	k (h^{-1})		$t_{1/2}$ (h)	
	ISF	Serum/Blood	ISF	Serum/Blood
Antibiotics				
Vancomycin ^a	0.53 ± 0.03	0.61 ± 0.17	1.3 ± 0.1	1.2 ± 0.3
Gentamicin	0.03 ± 0.02	0.33 ± 0.09	27.9 ± 22.7	2.2 ± 0.5
Immunosuppressants				
Tacrolimus	ND	0.12 ± 0.00	ND	5.9 ± 0.2
Cyclosporine	ND	0.32 ± 0.02	ND	2.2 ± 0.2
Mycophenolic acid	1.15 ± 0.65	1.22 ± 0.18	0.9 ± 0.6	0.6 ± 0.1
Anticonvulsants				
Valproic acid	0.15 ± 0.20	0.39 ± 0.35	9.8 ± 5.8	3.3 ± 2.5
Phenobarbital	0.03 ± 0.01	0.03 ± 0.00	27.9 ± 5.5	24.7 ± 3.8
Phenytoin	ND	0.28 ± 0.07	ND	2.6 ± 0.6
Chemotherapy				
Carboplatin	0.29 ± 0.28	0.03 ± 0.01	4.9 ± 3.5	21.9 ± 5.2
Cisplatin	0.13 ± 0.02	0.02 ± 0.01	5.6 ± 0.9	40.3 ± 21.3
Methotrexate	1.53 ± 0.46	1.76 ± 0.19	0.5 ± 0.2	0.4 ± 0.0
Miscellaneous				
Theophylline	0.73 ± 0.07	0.77 ± 0.12	0.9 ± 0.1	0.9 ± 0.2
Digoxin	1.48 ± 0.75	0.85 ± 0.65	1.3 ± 2.2	1.1 ± 0.5

Data are presented as mean ± SD. $n = 6$, except for vancomycin and gentamicin, where $n = 4$. ND, not detectable—below detection limit; ISF, interstitial fluid.

in ISF were (slightly) above the limit of quantitation (Fig. 1-E, Table 1).

Anticonvulsants: Concentration–Time Profiles in ISF and Blood

Valproic acid is a broad-spectrum anticonvulsant that is also indicated for headache, nerve pain, and a variety of psychiatric conditions.¹⁵ The dose of valproic acid (50 mg/kg) selected for this experiment was based on the published data on rabbits.¹⁶ Valproic acid ($n = 6$) exhibited markedly reduced C_{max} (178.2 ± 56.6 vs. $1992.5 \pm 217.6 \mu\text{mol/L}$) and AUC (177.5 ± 32.5 vs. $1424.9 \pm 213.3 \mu\text{mol h/L}$) in ISF compared with the blood ($p < 0.05$) (Table 1). An average delay in T_{max} of 0.27 h was observed in ISF. There was a nonparallel, biphasic decline in the blood concentration–time profile, which was also observed in ISF (Figs. 1-F1 and 1-F2, Table 2).

The barbiturate phenobarbital is used for generalized seizures or status epilepticus.¹⁷ The dose of phenobarbital (30 mg/kg) selected for this experiment was based on the published data on rabbits.¹⁸ Similar to valproic acid, the C_{max} (95.6 ± 16.8 vs. $239.3 \pm 20.1 \mu\text{mol/L}$) and AUC (2822.4 ± 274.5 vs. $6700.4 \pm 1199.2 \mu\text{mol h/L}$) of phenobarbital ($n = 6$) were reduced in ISF compared with the blood ($p < 0.05$) (Table 1). An average delay in T_{max} of 3.8 h was also observed in ISF. In contrast to valproic acid, the concentration–time curves of phenobarbital in the two matrices were similar, as evident by their parallel, uniphasic elimination characteristics exhibited in both matrices (Figs. 1-G1 and 1-G2, Table 2).

Phenytoin, a hydantoin, is also primarily used to treat generalized seizures and status epilepticus.¹⁷ The dose of phenytoin (10 mg/kg) selected for this experiment was based on the published data on rabbits.¹⁹ Like the immunosuppressant agents (tacrolimus, cyclosporine, and mycophenolate), phenytoin ($n = 6$) was not detected in ISF (Figs. 1-H1 and 1-H2, Table 1).

Chemotherapeutic Agents: Concentration–Time Profiles in ISF and Blood

Carboplatin, a platinum-based alkylating agent, is usually used in combination with other chemotherapy drugs for treating different types of cancer.^{20,21} The dose of carboplatin (18.7 mg/kg) selected for this experiment was based on the published data on rabbits.²² Carboplatin ($n = 6$) had dissimilar C_{max} (4110.5 ± 1058.9 vs. $3259.3 \pm 730.1 \mu\text{g/L}$) ($p < 0.05$), but similar AUC (5667.3 ± 1755.6 vs. $5004.1 \pm 2049.9 \mu\text{g h/L}$) ($p > 0.05$) in ISF compared with the serum (Table 1). An average delay in T_{max} of 0.26 h was observed in ISF. Despite similar PK parameters, there was a biphasic decline in both the serum and ISF concentration–time profiles, but the curves are not parallel, as evident by higher elimination rates in ISF (Figs. 1-I1 and 1-I2, Table 2).

Like carboplatin, cisplatin is an alkylating agent used in combination with other chemotherapy for a variety of cancers.^{20,21} The dose of cisplatin (3 mg/kg) selected for this experiment was based on the published data on rabbits.²³ In contrast to carboplatin, cisplatin ($n = 6$) had a similar C_{max} (5600.0 ± 1017.1 vs. $5639.0 \pm 799.9 \mu\text{g/L}$) ($p > 0.05$) but reduced AUC

(4002.3 ± 826.7 vs. $20128.7 \pm 1328.1 \mu\text{g h/L}$) ($p < 0.05$) in ISF compared with the serum (Table 1). An average delay in T_{max} of 0.24 h was also observed in ISF. Like carboplatin, the concentration–time curves of cisplatin in the two matrices were also dissimilar and can be characterized by a biphasic decline (Figs. 1-J1 and 1-J2, Table 2).

The antimetabolite methotrexate is primarily used in cancer, but has other indications such as rheumatoid arthritis and psoriasis.^{24,25} The dose of methotrexate (15 mg/kg) selected for this experiment was based on the reported data on rabbits.²⁶ Methotrexate ($n = 6$) exhibited reduced C_{max} (36.3 ± 8.7 vs. $90.8 \pm 10.9 \mu\text{mol/L}$) and AUC (19.7 ± 5.2 vs. $39.4 \pm 5.2 \mu\text{mol h/L}$) in ISF compared with the serum ($p < 0.05$). An average delay in T_{max} of 0.19 h was observed in ISF (Table 1). Unlike carboplatin and cisplatin, the concentration–time curves of methotrexate in the two matrices were similar and demonstrated a uniphasic decline (Figs. 1-K1 and 1-K2, Table 2).

Theophylline and Digoxin: Concentration–Time Profiles in ISF and Blood

The bronchodilator theophylline is a second- or third-line agent for treating asthma or chronic obstructive pulmonary disease.²⁷ The dose of theophylline (12 mg/kg) used in this study was selected from published data on rabbits.²⁸ Theophylline ($n = 6$) exhibited reduced C_{max} (62.6 ± 7.1 vs. $142.5 \pm 12.4 \mu\text{mol/L}$) and AUC (190.8 ± 51.8 vs. $513.9 \pm 103.7 \mu\text{mol h/L}$) in ISF compared with the blood, respectively ($p < 0.05$) (Table 1). An average delay in T_{max} of 0.42 h was observed in ISF. The concentration–time curves in the two matrices were similar, characterized by their parallel, uniphasic declines (Figs. 1-L1 and 1-L2, Table 2).

Digoxin, a cardiac glycoside, is second-line agent for atrial fibrillation or congestive heart failure.²⁹ The dose of digoxin (0.02 mg/kg) was selected from published data on rabbits.³⁰ Digoxin ($n = 6$) exhibited reduced C_{max} (5.9 ± 2.9 vs. $22.7 \pm 13.2 \text{nmol/L}$) and AUC (6.4 ± 3.2 vs. $14.6 \pm 6.9 \text{nmol h/L}$) in ISF compared with the serum ($p < 0.05$) (Table 1). An average delay in T_{max} of 0.44 h was observed in ISF. The serum concentration–time curve of digoxin can be characterized by a biphasic decline, but the ISF curve exhibited only a monophasic elimination (Figs. 1-M1 and 1-M2, Table 2).

Recovery

Drug recovery was performed for all 13 drugs and the results can be categorized in three groups: high recovery (70%–100%), low recovery (30%–70%), and marginal recovery (<30%) (Table 3). Tacrolimus, cyclosporine, and gentamicin showed marginal recovery and were excluded from the scoring algorithm (to be described in the *Discussion* section). Because the

Table 3. Recovery of Drugs after Passing Through the UF Probe ($n = 3$)

	Mean (%)	SD (%)	Adsorption
Vancomycin	75.8	15.6	No
Gentamycin	11.8	12.5	Yes
Tacrolimus	3.6	0.4	Yes
Cyclosporine	14.8	11.0	Yes
Mycophenolic acid	55.8	16.7	Yes
Valproic acid	95.7	3.9	No
Phenobarbital	89.6	1.1	No
Phenytoin	70.8	20.5	No
Carboplatin	99.8	4.1	No
Cisplatin	99.7	3.1	No
Methotrexate	93.5	1.7	No
Theophylline	100.5	1.5	No
Digoxin	89.0	3.6	No

molecular weight of all drugs was less than 5% of the MWCO of the UF membrane, low and marginal recovery rates are likely due to the adsorption and interaction processes between the drug and the membrane or tubing material of the probe.

DISCUSSION

Almost without exception, TDM requires drawing blood, which may be difficult in patients with fragile veins, neonates, infants, children, or the elderly. We are proposing to use ISF as an alternative matrix for TDM because it resembles blood plasma and can potentially be obtained noninvasively. As little information is available in the literature comparing PK parameters in blood and ISF, the current study aimed to characterize and compare drug concentration–time curves in the two matrices as a means to determine the suitability of ISF for TDM. Specifically, noncompartmental PK parameters (AUC, C_{max} , and T_{max}) were used to quantify the similarity and differences between the two matrices. To our knowledge, this is the first comprehensive, systematic analysis where a panel of drugs representing a wide variety of therapeutic classes for which TDM is warranted was tested in a single experimental setting.

The experiment was conducted in New Zealand White rabbits because of: (1) the availability of blood concentration data on this particular animal model that facilitated dose selection and data comparison, (2) the size of the animal which allowed for implantation of a relatively large (7 cm) UF probe for the collection of ISF, and (3) comparable protein binding and free fraction data compared with the humans. Blood concentrations observed in our animal model and those reported in literature on rabbits were in general agreement, providing support for the validity of our blood data. As examples, blood–concentration time profiles for cyclosporine by Awni and Sawchuk¹³ and valproic acid by Swanson et al.¹⁶ were similar

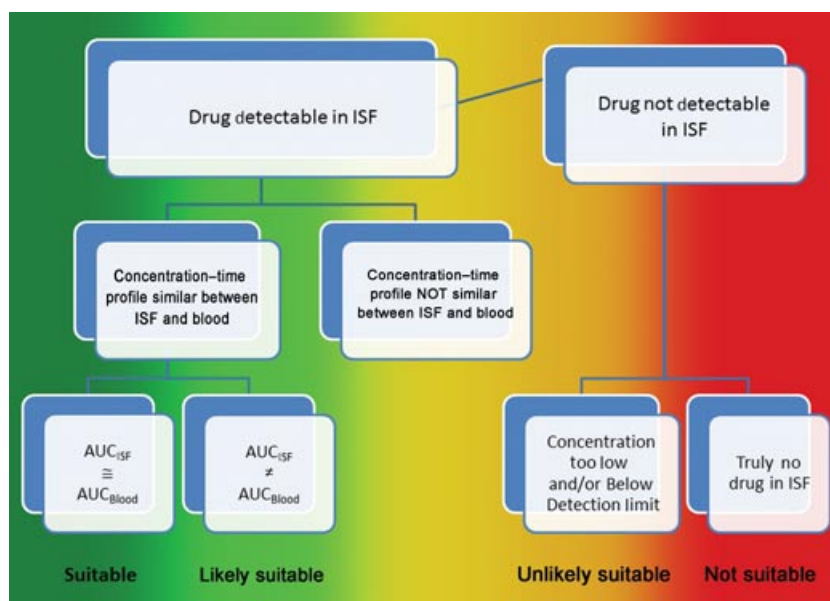


Figure 2. Scoring algorithm which helps in the determination of the feasibility of conducting TDM in ISF.

to our findings. As well, measurement of the glucose concentration equilibrium between ISF and blood is commonly reported in the literature.³¹ In our rabbit model, the glucose values were within $\pm 6\%$ of each other, as published previously in our pilot study.⁸

For each drug, the following criteria can be used to assess its suitability for TDM in ISF: (1) quantifiable drug levels in ISF, (2) comparable exposure (AUC) to blood, and (3) similar concentration–time profiles in both matrices (comparable terminal elimination rate constants as defined in *Materials and Methods* section). On the basis of these selection criteria, four categories were devised to rank order the feasibility of TDM in ISF (Fig. 2): (1) comparable exposure and similar concentration–time profile versus blood (directly suitable for TDM), (2) different exposure, but similar concentration–time profile versus blood (likely suitable for TDM), (3) detectable in ISF, but different concentration–time profile versus blood (unlikely suitable for TDM), and (4) not detected in ISF (not suitable for TDM). On the basis of these criteria, drugs tested in this study can be categorized as directly suitable (vancomycin), likely suitable (mycophenolate mofetil, phenobarbital, methotrexate, and theophylline), unlikely suitable (gentamicin, valproic acid, carboplatin, cisplatin, and digoxin), and not suitable (tacrolimus, cyclosporine, and phenytoin) for TDM in ISF (Table 4).

Vancomycin is the only drug tested in this study that could be categorized unequivocally as suitable for TDM in ISF because of comparable exposures and similar concentration–time profiles in both ISF and blood (Figs. 1-A1 and 1-A2). In contrast to rabbits, Caricato et al.⁵ reported reduced exposure of van-

comycin in ISF (collected from abdomen) compared with the blood in patients who suffered severe brain injury.⁵ However, because of the limited information available in the literature, further investigations are needed to determine whether the discrepancy in the reported AUC ratios can be attributed to altered vancomycin PK observed in different species or even physiological states (i.e., brain injury). Because of the linear elimination characteristic in humans (which is also observed in rabbits, Figs. 1-A1 and 1-A2) and reliable correlations between point concentrations and AUC, the usual practice in the clinic today is to obtain a single trough level of vancomycin for the purpose of TDM. As such, the similarity between ISF and blood C_{\min} observed in our model (Figs. 1-A1 and 1-A2) provides further support for using ISF for vancomycin concentration monitoring, but, as noted above, investigations are needed to confirm and validate this observation in humans. Replacing blood with ISF for TDM of vancomycin has potential significant impact to various aspects of patient care as vancomycin remains one of the most frequently monitored drugs today.

Drugs classified as “likely suitable” (mycophenolate mofetil, phenobarbital, methotrexate, and theophylline) exhibited similar elimination profiles between ISF and blood, despite differences in drug exposure. Unlike the categories of drugs to be discussed below, the similarity between the concentration–time curves in the two matrices increases the likelihood that PK relationships between the two compartments can be delineated with PK modeling. To our knowledge, our observations with mycophenolate mofetil and phenobarbital are novel as no other

Table 4. Feasibility Ranking for TDM in ISF

	Detectable in ISF?	Comparable AUCs?	<25% Difference Between Mean ISF versus Blood?	Ranking
Antibiotics				
Vancomycin	Yes	Yes	Yes	Suitable
Gentamicin	Yes	Yes	No	Unlikely suitable
Immunosuppressants				
Tacrolimus	No	No	No	Not suitable
Cyclosporine	No	No	No	Not suitable
Mycophenolate mofetil	Yes	No	Yes	Likely suitable
Anticonvulsants				
Valproic acid	Yes	No	No	Unlikely suitable
Phenobarbital	Yes	No	Yes	Likely suitable
Phenytoin	No	No	No	Not suitable
Chemotherapy				
Carboplatin	Yes	Yes	No	Unlikely suitable
Cisplatin	Yes	No	No	Unlikely suitable
Methotrexate	Yes	No	Yes	Likely suitable
Miscellaneous				
Theophylline	Yes	No	Yes	Likely suitable
Digoxin	Yes	No	No	Unlikely suitable

ISF, interstitial fluid; TDM, therapeutic drug monitoring. Drugs classified as “likely suitable”, “unlikely suitable” would require further characterization and/or pharmacokinetic modeling to ascertain their ultimate suitability status.

study has directly compared concentration–time profiles in ISF and blood, whereas the AUC ratios and concentration–time profiles of methotrexate and theophylline in ISF and blood were comparable between humans^{32,33} and data obtained in our rabbit model (Figs. 1-K and 1-L).

Drugs classified as “unlikely suitable” (valproic acid, gentamicin, carboplatin, cisplatin, and digoxin) exhibited different concentration–time profiles between ISF and blood. Specifically, reduced C_{\max} and delayed T_{\max} in ISF were generally observed, but it is the dissimilar elimination profiles characterized by different rate constants that make these drugs unlikely suitable for TDM in ISF. The general observation of delayed T_{\max} in ISF in our model (for all drugs that were detectable in the matrix) is consistent with that reported for glucose,⁴ but there is still debate whether this phenomenon is attributed to, or an artifact of, the measurement device.³⁴ In our study, delays in T_{\max} were still evident after subtracting the time needed for drugs to traverse the UF probe, suggesting that an artifact was unlikely the cause.

With respect to valproic acid, similar AUC ratios were also reported by Swanson et al.¹⁶ in rabbits (semen vs. plasma), and by Lindberger et al.³⁵ in humans [subcutaneous extracellular fluid (ECF), which consists of ISF and leaked plasma vs. plasma].³⁵ Consistent with our observation, gentamicin ISF and serum concentration–time profiles reported in another rabbit study³⁶ resembled those obtained in our experiment. A complicating factor for gentamicin in our model was the apparently low (~11%) percentage recovery in the ISF collection probe (Table 3). However, as gentamicin was readily detectable in ISF, one could

argue that low recovery would have affected only the magnitude, not the shape of the gentamicin concentration–time profile (i.e., the rate constants would have remained the same). This assumption needs to be tested in further experiments using other methods of ISF extraction.

Carboplatin data in the literature is less consistent and suggests species-related differences in drug exposure: carboplatin AUC in rat tumor ECF was similar to serum,³⁷ an observation shared in our rabbit model (Fig. 1-I) but not in humans (tissue ECF vs. serum).³⁸ On the contrary, similar cisplatin AUC ratios were found in primates (muscle ECF vs. plasma) and rats (tumor ECF vs. plasma),^{37,39} but not in our model (Fig. 1-J). The discrepancy in the reported AUC ratios might be explained by species-related differences in platinum tissue penetration and/or in the case of cisplatin, the presence of the platinum agent in other components of ECF (in rats and primates) that may have resulted in higher concentrations than that obtainable in ISF. With respect to digoxin, there are no reports directly comparing drug concentrations in ISF and serum, supporting the novelty of our data.

The antirejection agents (cyclosporine and tacrolimus) and the anticonvulsant phenytoin were not detected in ISF. Like gentamicin, the percentages of recovery of the antirejection agents in our ISF probe were generally low (Table 3), which could have led to the observation of no detection in this model. This assumption needs to be tested in future experiments using other methods of ISF extraction. To our knowledge, our results for the antirejection drugs are novel, as no literature data on animals or humans are yet available to support these observations. On the

contrary, a human study reported phenytoin levels in brain ECF, but found no correlation to phenytoin concentrations in blood.⁴⁰ However, as the study did not specifically report phenytoin concentrations in ISF, it is unclear what fraction of drug found in ECF could be attributed to the interstitium. An explanation for the lack of detection in ISF (other than inadequate drug recovery in the ISF probe discussed for the antirejection agents) could be the physiochemical characteristics shared by tacrolimus, cyclosporine, and phenytoin, which are all extensively bound to blood proteins and have relatively poor water solubility. As it is known that drug transport into ISF can be affected by protein binding,³ further investigations are needed to determine whether these properties were responsible for the absence of these drugs in rabbit ISF.

Our study investigated the suitability of ISF for TDM using a panel of 13 commonly monitored drugs in the clinic, and sets the stage for further systematic experiments (which are being planned) to examine the PK of drug transport into and out of ISF space. Despite various novel findings, a few limitations of this study and the associated future directions have been identified. These limitations include: (1) The PK parameters were characterized only after a single dose, whereas TDM is usually conducted at the point when the drug has reached steady state. As such, further experiments are needed to compare ISF and blood PK characteristics under steady-state conditions in the same model. (2) More rigorous sampling regimens are needed to allow compartmental PK modeling. This practice is warranted for drugs that are categorized as suitable or likely suitable for TDM in ISF (Table 4) so that PK relationships between blood and ISF compartments can be further elucidated. (3) In light of the potential site-related differences in ISF drug concentration,⁵ a systematic investigation is needed to ensure our ISF collection protocol (i.e., from rabbit neck) generates consistently reproducible results. (4) The data collected can be applied only to healthy rabbits of a specific age; thus, more experiments are warranted to determine the effects of physiological factors (e.g., age, weight, and sex) and disease states (e.g., renal and hepatic dysfunction) on the PK of drugs in ISF. (5) Only free drug concentration is measured in ISF; thus, future experiments need to take into account the free fraction or concentration of drugs in blood for the purpose of comparison and the effects of protein binding on drug transport into the ISF space. Further research into these questions will take some time, but will increase the fundamental understanding of drug transport between the blood and ISF compartment, and is expected to ultimately allow for more drugs being in the “suitable for ISF sampling” category. As well, the UF probe used in this animal experiment is not suitable for the clinic. A probe that is minimally

invasive and capable of collecting ISF from humans is under development for the purpose of planned future clinical studies.

CONCLUSIONS

Using a comprehensive panel of probes and a novel scoring algorithm, we have identified drugs that are suitable (vancomycin) or likely suitable (mycophenolate mofetil, phenobarbital, methotrexate, and theophylline) for TDM in ISF. Further studies are needed to take our observations with these drugs from the bench to the clinic (ultimately, whether a drug is suitable or not suitable in humans will be determined in the clinic). The ultimate goal is to eliminate the need for blood sampling for patients with fragile/“bad” veins, neonates, infants, and children for whom blood sampling is difficult.

ACKNOWLEDGMENTS

This study was funded by an operational grant from Collaborative Health Research Project and Natural Sciences and Engineering Research Council of Canada/Canadian Institute of Health Research. We thank Nilufar Partovi, Karen Ng, Morris Pudek, Catherine Halstead, and Frank Abbott for their help in obtaining the drugs and analyzing the samples for this study. We also acknowledge the excellent support from Gordon Gray, Rhonda Hildebrandt, and Kelly Kozevnikov at the Animal Care Centre and René G. Pedroza for help in measuring the drug recovery.

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