Predicting Hepatic Disposition Properties of Cationic Drugs Using a Physiologically Based, Agent-Oriented In Silico Liver

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Abstract

The In Silico Liver (ISL) plugs together autonomous software objects that represent hepatic components at different scales and levels of details. ISL parameters sensitive to drug-specific physicochemical properties (PCPs) were tuned so that ISL outflow profiles from a single ISL matched in situ perfused rat liver outflow profiles of sucrose and six cationic drugs. Antipyrine and diltiazem have the greatest degree of separation in PCP space of all pairs of the six compounds. Data for the other four, more closely spaced compounds comprised a training set for a simple artificial neural network (ANN) that was used to predict the PCP-sensitive, ISL parameter values for antipyrine and diltiazem given their PCPs. Those predicted parameter values were combined with the already-validated, drug-insensitive ISL parameter values. Simulation of the resulting ISLs gave expected liver perfusion outflow profiles for antipyrine and diltiazem that were within two-fold of the observed profiles.

1. INTRODUCTION

The In Silico Liver (ISL) [1], [2] (Fig. 1) is a first generation example of synthetic, agent-oriented and directed, physiologically based analogues that are intended for refining, exploring, and testing hypotheses about the details of hepatic drug disposition. Different objects represent hepatic components that vary in size, type, and function. Some represent molecules, others represent cells, and still others represent spatial aspects of hepatic organization and function. Drugs are represented as mobile objects and can be studied simultaneously or separately. Each ISL component can interact uniquely with drug objects that enter its local environment. The behavior of those drug objects is dictated by axioms, which specify relationships between drug properties, location, and proximity to other objects and agents. The consequences of simulated systemic and local interactions can be measured and studied simultaneously, analogous to how wet-lab experiments are conducted.

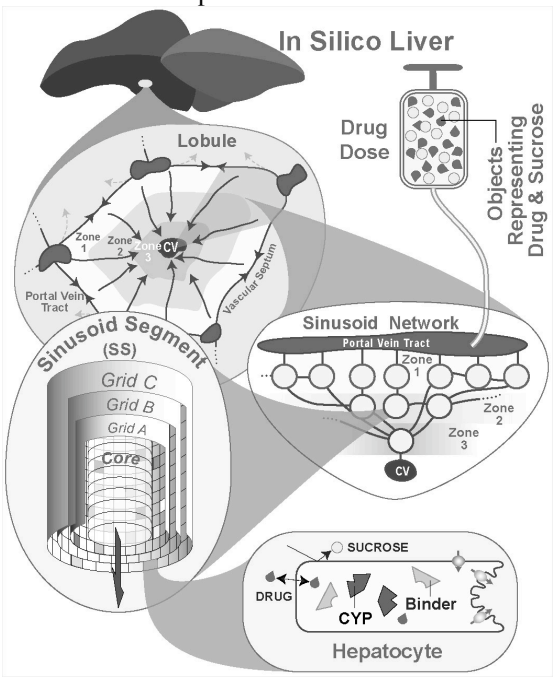

Figure 1. Illustrations of ISL model components. The six levels of ISL organization, from largest to smallest, are: 1) the ISL experiment system, 2) SINUSOID network, 3) SINUSOID segment, 4) SINUSOID spaces, 5) CELLS and EXTRACELLULAR domains, 6) ENZYMES and binders. Upper left: a schematic of a cross-section of a hepatic lobule. Blood flow enters lobules via terminal portal vein tracts, goes through sinusoids in three concentric zones, and drains into branches of a common central vein (CV). The three different zones have quantitative differences in structural characteristics and DRUG interaction capabilities. Upper right: objects representing a specific drug and sucrose are injected as a bolus into a simulated catheter that feeds into the portal vein tracts. Middle-right: a portion of the SINUSOID network is represented by an interconnected, three zone, directed graph. SINUSOID segments (SSs) are agents.

One is placed at each graph node: they simulate sinusoid functions. Lower-left: a schematic of SS component organization (SINUSOID spaces): Grids represent spaces and can contain objects representing the functions associated with a portion of SINUSOID space. DRUG and SUCROSE objects enter and exit a SS via the Core and Grid A. From Grid A, a COMPOUND can access the other spaces. Grids govern their interaction with mobile COMPOUNDS based on the location of the COMPOUND in the grid. The potential for heterogeneous properties within different grids is illustrated by different shadings of grid locations. Objects functioning as containers (for other objects) are used to represent cells, and can be assigned to any grid location. The Core represents blood flow; Grid A represents the sinusoid rim; Grid B represents endothelial cells and fenestra. The CELLS in Grid B are agents. Grid C represents all other spaces, including the Space of Dissé and hepatocytes. A HEPATOCYTE container is an agent: objects representing all needed intracellular components can be placed within and managed by the HEPATOCYTE agent. All enzymes that metabolize drug are represented by ENZYME objects (e.g., CYP); cell components that simply bind, sequester, and release drugs are represented by BINDERS. Additional binders representing transporters and other capabilities can be easily added as needed. Cell containers can distinguish between DRUG and SUCROSE; they do not allow SUCROSE to enter. Bile was not needed for these simulations, but it can be represented easily as an extension.

ISLs have been validated by successfully simulating the hepatic disposition properties of atenolol, antipyrine, labetalol, propranolol, prazosin, and diltiazem, along with coadministered sucrose. One, common parameterized ISL structure was used for all ([2] and unpublished results). Those results supported two hypotheses. 1) The mappings in Fig. 2 between ISL components and their arrangements, and corresponding hepatic components and their arrangements are sufficiently realistic to simulate hepatic outflow profiles that are good matches to referent data. 2) The simulated drug-ISL-component interaction events map to corresponding hepatic disposition events.

Differences in the physicochemical properties (PCPs) of drugs are responsible for differences in how they interact with hepatic components. Those differences are responsible for each drug's characteristic hepatic disposition properties. To the extent that the hypothesized mappings B and C in Fig. 2 are true, a quantifiable mapping should exist between tuned ISL parameter values and the set of PCPs that are most influential in drug-hepatic component interactions. If we can discover a plausible, quantifiable mapping, then it may prove useful in providing quantitative predictions of ISL parameter values for compounds for which the hepatic disposition properties are not yet available. Simulations using the ISLs so parameterized would then stand as predictions of expected hepatic disposition properties for the targeted compound. Current prediction methods rely on sophisticated correlation methods; those that are within 2-fold of actual values are considered acceptable [3].

An artificial neural network (ANN) was used to establish a predictive relationship between the PCP-sensitive, ISL parameter values and the corresponding PCPs for atenolol, labetalol, prazosin, and propranolol (Table 1). PCP-sensitive, ISL parameter values were then predicted for antipyrine and diltiazem using the trained ANN and each compound's PCPs as inputs. Those values were then combined with the already-validated, drug-insensitive ISL parameter values. Simulation of the resulting ISLs gave expected liver perfusion outflow profiles for antipyrine and diltiazem that were within two-fold of the observed profiles.

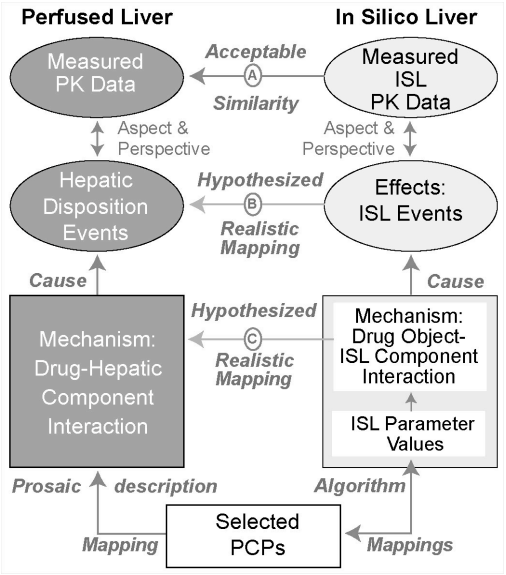

Figure 2. Illustration of relationships between ISL mechanisms and its components, along with relationships between the ISL and referent, perfused liver counterparts. To the extent that mappings A, B and C are realistic, the relationship between a drug's physicochemical properties (PCPs) and specific hepatic disposition events will have an ISL counterpart. Discovering plausible methods to make a quantitative mapping between a drug's PCP and PCP-sensitive ISL parameter values is expected to enable prediction of DRUG–specific, PCP-sensitive ISL parameter values, given only a new drug's PCPs. ISL simulations using those predicted parameter values stand as a prediction of the expected disposition behavior of that new drug.

2. METHODS

To clearly distinguish in silico components and processes from corresponding hepatic structures and processes, we use SMALL CAPS when referring to the former.

2.1 Argument for an Agent-Oriented Approach

As stated in [1], a long-term goal is to produce increasing similarity between measurable ISL attributes following administration of a drug, and corresponding, measured hepatic attributes. To reach that goal we engineered the ISL to ex-

hibit the following capabilities (adapted from [1])

1. *Turing test*: To domain experts (in a type of Turing test), ISL data are indistinguishable from their perfused liver counterparts. So doing has provided validation evidence and required that ISLs be suitable for experimentation.
2. *Phenotype overlap*: ISL attributes overlap those of perfused liver systems. Similar attribute measures are used to document ISLs and perfused livers. As ISLs evolve, measurements of them during execution have increasingly overlapped corresponding measures of perfused liver experiments.
3. *Mappings*: Observables at any ISL level, whether measured or not, are designed to be consistent with those of the referent system. Doing so has enabled documenting iteratively concretizable mappings between referent and in silico components and mechanisms.
4. *Local mechanisms*: Behaviors that emerge during a simulation are the consequences of local (as opposed to merely reactive) mechanisms—local component interactions.
5. *Transparency*: Simulation details, as they unfold, are visualizable, measurable, and comparable where feasible to those of the wet-lab, perfused liver systems.
6. *Adaptability*: In addition to flexibility and reusability, ISL components are constructed to be adapted to function as components in other physiologically based models, such as whole organism models.
7. *Reusability*: ISL components have been designed to be autonomous and thus can be reconfigured to represent other tissue components, experimental conditions, and compounds, *alone or in combination.*
8. *Flexibility*: It is relatively simple to increase or decrease detail in order to simulate an additional phenotypic attribute or change usage and assumptions, without requiring significant reengineering. Achieving this capability is enabling cycles of testing and ISL refinement.
9. *Components articulate*: ISL attributes are caused by interactions of autonomous components. To study alternative mechanisms it must be easy to join, disconnect, and replace components without having to reengineer the ISL or its components.

Everything except *Local mechanisms* can be achieved without agents. *Local mechanisms* and *Adaptability* both strongly suggest using an agent-oriented approach, which we have done. *Phenotype overlap*, *Mappings*, *Transparency*, *Reusability*, *Flexibility*, and *Articulation* only weakly suggest agency but strongly suggest that the modeling approach be object-oriented, which it is.

2.2 ISL Design and Construction

ISL structure (Fig.1) for this project is as detailed in [1] and [2]. For convenience, a brief description follows. An ISL is agent-oriented; in silico experiments are agent-directed. We assumed that hepatic lobule function is similar throughout the liver. We therefore represent the entire liver as a large collection of similar lobules. Each lobule is represented as an interconnected network of sinusoids. Blood flow is represented as entering portal vein tracts and vascular septa, and draining into a common central vein (CV). Flow patterns are represented by an interconnected directed graph. Objects representing sinusoidal spaces and function, along with related spatial features, are placed at each graph node. Within the ISL, those objects are software agents called Sinusoidal Segments (SSs).

Table 1. Physicochemical properties SUCROSE (SUC), ATENOLOL (ATEN), ANTIPYRINE (ANTI), PRAZOSIN (PRAZ), LABETALOL (LABETA), PROPRANOLOL (PROP), and DILTIAZEM (DILT)

Category	Parameters	SUC	ATEN	ANTI	PRAZ	LABETA	PROP	DILT
I: Size	MW	342.3	266.3	188.2	383.4	328.4	259.4	414.5
	Volume	283.6	260.9	178.3	336.0	314.8	257.8	377.8
II: Partitioning	$\log P_{app}$ [a]	-3.7	0.14	0.33	1.88	2.69	3.10	3.53
III: Binding	f_{uB} [b]	-	0.74	0.60	0.54	0.52	0.45	0.28
IV: Topology related	RB count [c]	5	8	1	4	8	6	7
	TPSA [d]	189.5	84.6	26.9	107.0	95.6	41.5	59.1
V: Hydrogen bound related	HBD count [e]	8	3	0	1	4	2	0
	HBA count [f]	11	4	2	8	4	3	5
	Tautomer count [g]	1	2	0	3	7	0	0

[a] $\log P_{app}$: log of the octanol-water partition coefficient at pH 7.4, 37ºC; [b] f_{uB}: Unbound fraction of each drugs in the liver perfusion medium; [c] RB: number of rotatable bonds, a measure of molecular flexibility; amine C-N bonds are not considered because of their high rotational energy barrier; [d] TPSA: topological polar surface area: sum of fragment contributions; O- and N- centered polar fragments are considered; [e] HBD: hydrogen bond donor; [f] HBA: hydrogen bond acceptor; [g] Tautomers are organic compounds that are interconvertible by tautomerization

SS design and construction has been validated [2]. A SS is modeled as a discretized, tube-like structure comprised of a blood "Core" surrounded by three identical size spaces. Each space is modeled as a 2D grid. Together they simulate a 3D structure. Grid A represents sinusoid edges near endothelial cells. Grid B represents the endothelial layer. Grid C represents the Space of Disse and hepatocytes. Two SS classes are specified: S1 and S2. Compared to a S2, S1 have a shorter internal path length and smaller surface-to-volume ratios. When SSs are placed randomly at graph nodes, the resulting structure simulates the heterogeneity of sinusoid network. A lobule's interior is represented by three concentric zones to distinguish the quantitative difference in intralobular structural characteristics and enzymatic and transporter gradients. A lobule's blood supply feeds into several sinusoids that merge in stages to only a small fraction of their original number as blood is fed into the CV. Therefore the number of directed graph nodes per zone is always in the order of Zone 1 > Zone 2 > Zone 3. Because interconnections between sinusoids are frequent in the periportal region and are rare near the CV, there are more interconnections between Zone 1 nodes than between Zone 2 nodes, and no interconnections between Zone 3 nodes. The complete set of parameters that specify lobule form, function, and other features are listed in [2]. Many of those parameters are stochastic to simulate uncertainty and biological variability.

2.3 Simulation of Hepatic Drug Disposition

ISL experiments follow the same protocols used in situ. COMPOUNDS are objects that are in silico analogues of sucrose, an extracellular space marker, and six cationic drugs: antipyrine, atenolol, prazosin, labetalol, propranolol, and diltiazem. Wet-lab experiments: a bolus containing similar amounts of sucrose and drug was injected into the portal vein through a catheter. Perfusate was collected using a fraction collector. The fraction of the administrated dose contained per collection interval was measured.

In the ISL, each COMPOUND is represented using an object that carries identifying information (including values of PCPs). They move through the LOBULE and interact with each SS feature encountered. A COMPOUND's behavior is determined by the information that it carries, along with the LOBULE and SS features encountered during its unique trek from portal vein to CV. During a simulation cycle, an encountered ISL component can "read" the information carried and use it in its prespecified logic to customize its response, in compliance with its parameter values (PV), following some.

COMPOUNDS enter LOBULES via the portal vein. After that, they enter a SS in Zone 1. Simulated flow occurs only in the Core. Until being collected at the CV, each COMPOUND has several stochastic options. The parameter *CoreFlowRate* simulates blood flow. The parameter *SinusoidTurbo*, which simulates turbulent flow, biases COMPOUND movement in the three spaces in the CV direction. In the Core or Grid A, a COMPOUND can move within either space, jump to an adjacent space, or exit the SS. Within Grid B, it has three stochastic options: move within Grid B, jump back to Grid A, or on to Grid C. A DRUG but not SUCROSE can move into CELLS in either Grid B or C. A COMPOUND can exit a SS only from Grid A or the Core. After a COMPOUND exits a SS in Zone 3, it enters the CV: its arrival is recorded, simulating being collected by a fraction collector. An animated visualization of those events during a simulation at both individual ISL and SS levels for SUCROSE and ANTIPYRINE administrated together is available through Supplement Data of [2].

An unspecified number of cells are represented by objects called CELLS in Grids B and C. They are placed randomly at some fraction of the available grid locations in Grids B and C at the start of each simulation. They function as containers for other objects. BINDERS are the INTRACELLULAR components that represent transporters, enzymes, and other cellular material that binds or sequesters drug molecules. A binder within a Grid B CELL can only bind and later release a DRUG. A binder within a Grid C HEPATOCYTE can bind DRUG and either release or METABOLIZE it.

Of the 38 ISL parameters, 28 control LOBULE and simulation features that are independent of administrated COMPOUND. The ten in Table 2 are sensitive to differences in PCPs; five of the ten are used only for COMPOUNDS that can enter CELLS. We located an ISL parameter domain capable of generating biologically realistic outflow profiles. We tuned the ISL so that outflow profiles of SUCROSE and four of the cationic DRUGS, ATENOLOL, LABETALOL, PRAZOSIN, and PROPRANOLOL, were judged similar to referent profiles based on Similarity Measure values. See [2] for example matches.

For this report, the goal has been to establish a quantitative mapping from the PCPs of the above four compounds to the set of COMPOUND-specific ISL parameter values. We then used that mapping to predict ISL parameter values for ANTIPYRINE and DILTIAZEM, given their PCPs, and then used those values to parameterize a unique ISL. The resulting simulations stand as predictions of the hepatic outflow and disposition properties for those two compounds.

2.4 Prediction of ISL Parameter Values: Artificial Neural Network (ANN)

The ISL has three parameter categories: those that 1) control lobule graph and 2) sinusoid structures are the same for all drugs, plus 3) those that control the PCP-sensitive parameters. We need only predict the latter. To establish a quantitative, nonlinear mapping, we used a simple feed-forward ANN [4], recognizing that the small training set would lead to an over-fitted prediction. Nevertheless, we

Table 2: Tuned PCP-sensitive ISL parameter values for ATENOLOL, PRAZOSIN, LABETALOL, and PROPRANOLOL

ISL Attribute or Event	ISL Parameters	ATENOLOL	PRAZOSIN	LABETALOL	PROPRANOLOL
A. Effective DRUG size	*ISL2WetLabScaling*	7	4.6	6	2.8
B. Movement between spaces	*A2BJumpProb*	0.1	0.62	0.35	0.75
	B2AJumpProb	0.6	0.29	0.2	0.21
	B2CJumpProb	0.3	0.5	0.5	0.5
	C2BJumpProb	0.6	0.42	0.5	0.32
C. Binding to LOBULAR components	*BindersPerCellMin*	5	8	5	8
	BindersPerCellMax	10	15	10	17
	SoluteBindingProb	0.35	0.43	0.6	0.42
	SoluteBindingCycles	25	24	25	23
D. METABOLISM	*MetabolizeProb*	0.35	0.19	0.3	0.09

used it because the method is known to be good at finding patterns and establishing quantitative relationships between data sets based on those patterns. We used a backpropagation training algorithm that used normalized values of the four sets of nine PCPs in Table 1 as inputs; values of the four sets of ten ISL PVs in Table 2 were obtained as outputs. We then used the trained ANN to separately predict a full set of ten PCP-sensitive, ISL PVs for ANTIPYRINE and DILTIAZEM, given their nine PCPs as inputs.

Values were normalized by dividing each point by its vector norm. E.g., if drug (point) X has PCPs A, B and C, then it was divided by its length N, i.e., $N^2 = A^2 + B^2 + C^2$ and $X_{normalized} = (A/N, B/N, C/N)$. The output values were normalized in the same way. Consequently, the predictions too were normalized; to be used, the predictions had to be scaled: multiplied by the norm of training vector.

2.5 Similarity Measure.

The Similarity Measure (SM) used to compare ISL with the referent outflow profiles is detailed and discussed in [1] and [2]. Briefly, we assumed that the coefficients of variation of observations between t = 1 and t = 100 seconds from repeat experiments are constant. For each referent outflow profile measure P, we create two curves, $P^l = P(1 - d)$ and $P^u = P(1 + d)$. They form lower and upper bounds of a band around P. Here, we used d = 1 standard deviation (SD) for sucrose from six repeated in situ experiments and the mean value for a given collection interval, pooled over all collection intervals. The relative variability in repeat measures for each of the compounds was assumed to be the same. The SM value is the fraction of evenly spaced, outflow profile measures falling within the band. An ISL outflow profile was deemed similar to the referent if 80% or more of ISL outflow values were within the band (SM ≥ 0.8). Pharmacokinetic predictions that are within a factor of two of actual values are generally considered acceptable [3]. We did the same. Nevertheless, we calculated SM values using two reference bands: d = 1 and 1.5.

2.6 The Framework and In Silico Experimental Method.

The means for generating and managing all of the above components and capabilities are organized within the framework diagrammed in Fig. 3. They are managed in part by an Experiment Agent that fills a role similar to that of a researcher conducting and recording the results of wet-lab experiments. Additional details are provided in the Supplementary Material of [1].

The in silico experimental method is as follows. An experiment is set up by building the software into an executable, creating/editing the parameter vector, and beginning the simulation. An experiment consists of several runs that comprise a Monte Carlo style simulation, the results of which are post-processed to arrive at a nominal data set that represents the result of the experiment. The parameter vector also contains the limited capability to specify multiple experiments by iterating over some of its values. The raw data is preserved, but this is typically used only for parameter sweeps and data matching. At the end of an experiment, the data is isolated with the "make" target and one of several data reduction scripts may be run to process and analyze the simulation result. Typically, this processing involves an external (to the simulation) calculation of the similarity measure (SM) and plots made for examination by the experimenter. A new parameter vector is then designed based on the quality of similarity and the profile features as indicated by the data processing scripts.

2.7 ISL Framework and Execution.

Code is managed using CVS with a single HEAD branch and ChangeLogs for each commit. Experiments are conducted using the last stable version of the HEAD branch. Validation data is loaded using HDF5 (a Hierarchical Data

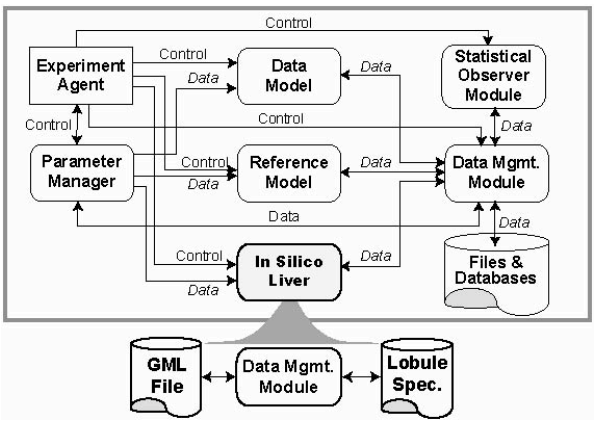

Figure 3. Framework of the ISL (In Silico Liver) showing key components. The Experiment Agent manages the three models, each of which is an agent responsible for its own contents. Of the three, the in silico liver in Fig. 1 is the only agent composed of sub-agents: the SS and the CELLS in Grids B and C.

Format product). As an experiment runs, simulation data is kept in memory (indexed by Scheme representation of the parameter vector) until the end of the experiment, at which point it is written to comma separated files indexed by filename. A "make" target is used to move all the relevant input, output, and parameter data into a dated directory, preserving the integrity of the relationships. A Python script processes the raw output and prepares the data for analysis and plotting by R scripts.

Development follows a loose and iterative specification, implementation, test, and commit process. Each specific change is documented in the ChangeLog. The code is tested against a brief experiment where inputs, parameters, and output are compared with prior experiments and examined for anomalies. The changes are then committed to CVS; there is one CVS module for the entire framework.

Coding standards follow typical Swarm patterns, using Objective-C and C++ (with Object Oriented patterns). Development adheres to the architecture of the FURM method (http://furm.org). There are seven sub-modules: one for the experimental data; one for the Reference model; one for the Research models (ISLs); one for documentation; one for common modeling utilities (programming generics, graphs, and the statistics and data management classes); one for the input data; and one for the set of data processing scripts and other tools.

Experiments were executed on an eight node OSCAR cluster (oscar.openclustergroup.org/) running RedHat's Fedora 5. Run distributions used MPICH 1.2.7 (www-unix.mcs.anl.gov/mpi/mpich1/). The ISL was compiled using GCC 4.1.1 against the Swarm 2.2.3 Objective-C libraries (swarm.org/wiki/Main_Page). The initial PRN seed was extracted from the machine's clock. Each completed experiment was archived and logged with the date and time using a Makefile target. Examination and processing of data from simulations used a combination of Matlab (7.0.0) and R (2.4.0) (www.r-project.org/) scripts, and Microsoft Excel. As done previously [1], [2], we smoothed results from each simulation experiment using the Smodulus_smoothing function from the Rwave (version 1.22) package of R. For the results presented, a wavelet window of three observations at 0.5-second intervals was used.

3. RESULTS

Table 3 lists the ISL PVs for ANTIPYRINE and DILTIAZEM predicted using nine ANN inputs (PCPs) and ten outputs (ISL PVs).

Table 3. Predicted parameter values for ANTIPYRINE and DILTIAZEM that were combined with PCP-insensitive parameter values to generate the outflow profiles in Fig. 4.

ISL parameter values	ANTIPYRINE	DILTIAZEM
ISL2WetLabScaling	5.0	1.0
A2BJumpProb	0.53	0.75
B2AJumpProb	0.51	0.07
B2CJumpProb	0.35	0.5
C2BJumpProb	0.47	0.15
MetabolizeProb	0.27	0.02
BindersPerCellMin	7	8
BindersPerCellMax	15	17
SoluteBindingProb	0.12	0.39
SoluteBindingCycles	21	23
SM values (±1 SD)	0.68	0.71
SM values (±1.5 SD)	0.97	0.98

We used the predicted ISL PVs in Table 3 along with the previously specified [2], PCP-independent, parameter values to parameterize new ISLs for ANTIPYRINE and DILTIAZEM. Resulting simulations the expected outflow profiles presented in Fig. 4. The ±1 SD and ±1.5 SD SM values are a factor of 0.68 and 0.97 for the observed wet-lab antipyrine data and 0.71 and 0.98 for the observed diltiazem data. One simulated outflow profile is the result of pooling 48 separate Monte-Carlo trials. One trial represents a single lobule and 48 trials simulate the whole liver. The structural and microdearchitectural details of the ISL used for each of the 48 simulation trials per experiment were nondeterministic.

4. DISCUSSION

Our approach to gaining insight into the complexities of pharmacology is to develop and study agent-oriented analogues of the systems used in research. Our expectation is that, when many of the measurable phenotypic attributes of ISLs are similar to corresponding phenotypic attributes of the experimental system, the mechanisms of the analogue will stand as dynamic, viewable hypotheses of the mechanisms of interest in the referent system (Fig. 2).

When conducting experiments on living objects, it is tempting to limit attention to those living objects. However, the experimental system also includes the immediate environment of the living component, and that includes the devices that keep it alive and that are used to measure it. If they change, the measures of the living object's behaviors may also change. Should the researcher external to the experimental system be considered part of it? If the latter, the boundaries of the referent system should be extended to include the researcher. If s/he is changed, behavior measures may also change: exact duplication of results by independent researchers has always been the science ideal. Results that satisfice are the norm. With these considerations in mind, we have designed our analogue ISL system so that it includes not only an autonomous analogue representing the living object, but also an analogue of the laboratory items used to keep it alive and that are essential to experiment execution, and, importantly, an Experiment Agent. The latter is an analogue of the researcher observing and recording measures. The scientist manages and directs the experiment. S/he can even choose to intervene, given some observation. The autonomous Experiment Agent has been designed to have the same capabilities as they are needed.

There is a broad consensus [5] that one can expedite effective decision-making concerning new drug candidates undergoing development when reasonably reliable predictions can be made available of the candidate drug's pharmacokinetic properties. The currently used, physiologically based pharmacokinetic models are differential equation based. Their PCP-sensitive parameters purposefully conflate drug PCPs with features and properties of the biology, such as aspects of hepatic histology and intracellular components that can bind or sequester drugs. That modeling and simulation approach carries with it considerable risk of obscuring the "causal basis" of important pharmacokinetic properties. The ISL, on the other hand, has a collection of simple mechanisms assembled from finer-grained components. It has precise control over conflation. The causal basis is there in the drug-component interaction logic. Our expectation has been that at some level of granularity, the complexity will be sufficiently unraveled so that the logic for a given ISL component-drug-interaction, its "causal basis," will rely heavily on just a subset of easily specified drug PCPs and measurable biological features. In some cases, information about the consequences of a compound interacting with a hepatic component such as membrane transporter may be represented adequately within the logic by just a few PCPs and/or molecular descriptors.

The degree of separation between antipyrine and diltiazem in nine-dimensional PCP space is greatest of the six cationic drugs in this study. To illustrate, antipyrine has the smallest molecular weight, volume, and 2nd to the smallest logP_{app}, whereas diltiazem has the largest values of those three PCPs (Table 1). We speculated that predicting their disposition properties, starting with their PCPs and the validated, ISL parameterizations for the four other COMPOUNDS,

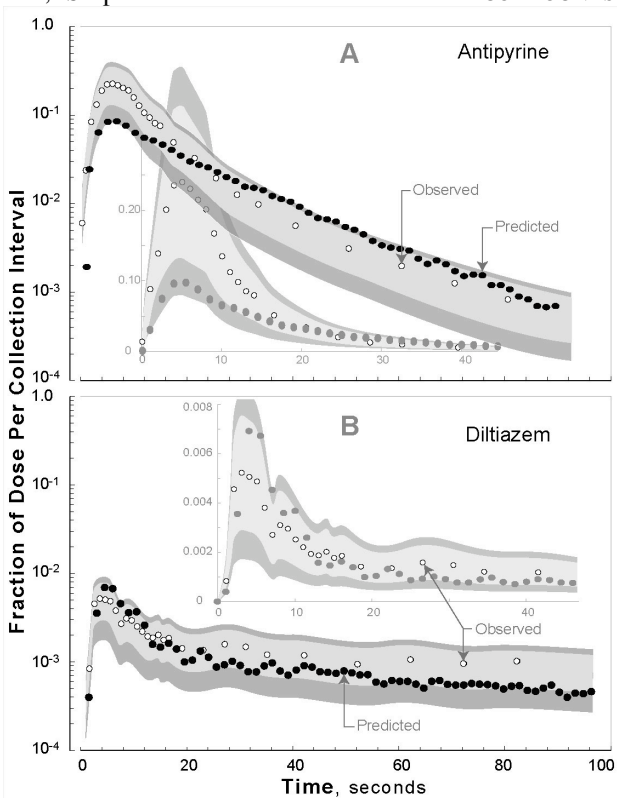

Figure 4. Expected outflow profiles (black circles) for antipyrine (**A**) and diltiazem (**B**) using predicted parameter values. These plots show results of simulation experiments for which the PCP-sensitive PVs listed in Table 3; they were predicted using the ANN, as described in the text. All other ISL parameter values were identical to those reported in [2]. Open circles: observed, referent wet-lab data. The data are the fraction of dose per outflow unit (per ml for the referent) as a function of time (1 simulation time unit = 1 second) after dosing with DRUG. The light gray band spans the range for the mean of the referent data ± one standard deviation (SD) as specified in the text. The dark gray band spans the range for the mean of the referent data ± 1.5 SD. Each ISL datum is the smoothed (window size: 3 values) mean value of 48 independent ISL runs.

would be challenging for any modeling and simulation method. The ANN predicted ISL parameter values gave rise to predicted profiles that were well within a factor of two of the actual profiles. The results support the case that the differences in the tuned values of the ten PCP-sensitive ISL parameters could be traced primarily to differences in PCPs. The results also support our vision that an ISL parameter estimation method, which uses PCPs to qualify the relative differences between compound-specific behaviors at the level of detail represented by its mechanisms, can provide a parameterized ISL, and the simulations in turn can provide reasonable, ballpark estimates of the expected dis-

position properties of the target compound. That bodes well for using the ISL class of models for predicting pharmacokinetic properties, given only molecular structure information. Being able to predict the hepatic disposition properties of two quite different compounds using a training set that had PCPs between those of two target compounds also suggests that the ISL features, and the granularity of ISL mechanisms are adequate for the prediction task. It is important to note, however, that the six drugs in this study are all weak bases. As such, they occupy a common sub-region of PCP space. More drug cases, spanning additional regions of PCP space, will be needed to build confidence that the above, apparently favorable parameter prediction situation is a consequence of the synthetic method and the ISL, rather than fortuity.

Although the ANTIPYRINE simulations in Fig. 4 (using the predicted parameters) met the quantitative criterion for the similarity measure, it does not meet the "eyeball check." The relationship of the peak to the later, more log-linear portion of the referent, wet-lab antipyrine data is clearly different than the corresponding relationship for the simulated ANTIPYRINE data. The character of the wet-lab antipyrine profile is fundamentally different from that of the wet-lab diltiazem profile. The particular parameter values chosen by the ANN for DILTIAZEM exhibit that character better than do those selected for ANTIPYRINE. We need to better understand the underlying causes of such features in order to improve the prediction methods.

Because of the small training set, the ANN, although simple, was overparameterized. We need to explore other prediction options (the Fuzzy c-means algorithm may be such an option) that can be used even with such sparse data. Because of R&D development costs, sparse data is a constant reality within the new drug development context.

5. CONCLUSION

Because it is a synthetic model, the ISL successfully shrinks the PCP-sensitive phenomenal manifold relative to that of tightly coupled, mathematical, PK models. Those traditional, equation-based models reduce the phenomenal manifold to a set of real-valued parameters interacting within and assuming a relational continuum. Although one can reduce the number of parameters being considered with these models, in order to maintain accurately quantified relationships, the set of reduced parameters must still accurately capture the aggregated relationships of the larger parameter set. This implies that, for tightly coupled PK models, the phenomenal manifold remains just as complex even through there are fewer parameters in the PK equations. The fine-grained relationships that traditional pharmacokinetic models conflate into mathematical parameters are necessarily embedded in the coarse-grained relationships of these parameters. In contrast, the ISL can actually be simplified because it is an assemblage of coupled, abstract mechanisms, such as transfer between spaces and entry into cells, where the finer-grained relationships can be completely ignored. We posit that, by having simplified, yet fine-grained, heuristic models, like the ISL, which are commensurate with relatively simple parameter estimation methods, the biological mechanisms become more understandable and accessible. Increased accessibility can provide the methodological leverage needed to build synthetic analogues that will enable us to explore, digest, categorize, understand, and use the massive amounts of data being generated by "omic" technologies.

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REFERENCES

[1] Hunt CA, Ropella GE, Yan L, Hung DY and Roberts MS (2006) Physiologically based synthetic models of hepatic disposition. *J Pharmacokinet Pharmacodyn* 33: 737-72.

[2] Yan L, Ropella GEP, Park S, Roberts MS and Hunt CA (2007) Modeling and Simulation of Hepatic Drug Disposition Using a Physiologically Based, Multi-Agent In Silico Liver. *Pharm Res* Published online: 28 November 2007 (DOI: 10.1007/s11095-007-9494-y).

[3] Fagerholm U. (2007) Prediction of human pharmacokinetics—evaluation of methods for prediction of hepatic metabolic clearance. *J Pharm Pharmacol* 59: 803-28.

[4] Hudson DL and Cohen ME (1999) *Neural Networks and Artificial Intelligence for Biomedical Engineering (IEEE Press Series on Biomed Engineering)*. Wiley-IEEE Press.

[5] Theil FP, Guentert TW, Haddad S, Poulin P (2003) Utility of physiologically based pharmacokinetic models to drug development and rational drug discovery candidate selection. *Toxicol Lett* 138: 29-49.

[6] Hung DY, Chang P, Weiss M and Roberts MS (2001) Structure-hepatic disposition relationships for cationic drugs in isolated perfused rat livers: transmembrane exchange and cytoplasmic binding process. *J Pharmacol Exp Ther* 297:780-789.

[7] Yan L, Sheihk-Bahaei S, Park S, Ropella GEP and Hunt CA (2008) Predictions of Hepatic Disposition Properties Using a Mechanistically Realistic, Physiologically Based Model. *Drug Metab Dispos* doi:10.1124/dmd.107.019067.