Computational Strategies Unravel and Trace How Liver Disease Changes Hepatic Drug Disposition

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Abbreviations:

CV: central hepatic vein; ISL: In Silico Liver; OOP: object-oriented program(ming); PCP: physicochemical property; PK: pharmacokinetic; PV: terminal portal vein tracts; SM: Similarity Measure; SS: Sinusoidal Segment
Abstract

Liver disease changes the disposition properties of drugs, complicating drug therapy management. We present normal and “diseased” versions of an abstract, agent-oriented In Silico Liver (ISL), and validate their mechanisms against disposition data from perfused normal and diseased rat livers. Dynamic tracing features enabled spatiotemporal tracing of differences in dispositional events for diltiazem and sucrose across five levels, including interactions with representations of lobular microarchitectural features, cells, and intracellular factors that sequester and metabolize. Differences in attributes map to measures of histopathology. We measured disease caused differences in local, intralobular ISL effects, obtaining heretofore-unavailable views of how and where hepatic drug disposition may differ in normal and diseased rat livers from diltiazem’s perspective. Exploration of disposition in less and more advanced stages of disease is feasible. The approach and technology represent an important step toward unraveling the complex changes from normal to disease states and their influences on drug disposition.
Introduction

Changes in drug disposition properties caused by liver disease complicate drug selection and therapy management. Recent advances in computational biology are providing new, broadly applicable strategies to unravel such complexities. How does cirrhosis (Hung et al., 2002a,b) cause observed changes in hepatic drug disposition? More detailed understanding of precisely how drugs behave in diseased relative to normal livers requires new modeling strategies in which events at different levels can be observed and measured in both space and time. We address this need and provide a novel means of creating and testing real, working representations of hypothesized mechanisms. We present and validate a plausible explanation for structural and functional differences in the normal and diseased rat livers from the perspective of a specific drug, diltiazem (Hung et al., 2001), a cationic compound known to interact with hepatic components at all levels. We then show how those changes may have caused the observed differences in diltiazem’s disposition.

The In Silico Liver (ISL) (Hunt et al., 2006), (Yan et al., 2008a,b) in Fig. 1 is an abstract analogue built in software using discrete, object-oriented methods. It is an advanced example of what has been referred to as executable biology (Fisher and Henzinger 2007), (Hunt et al., 2008). ISLs are physiologically based, multi-level analogues of livers undergoing perfusion. They are not intended to be accurate, precise descriptions of how we think the liver works nor are they intended to provide precise, accurate predictions. Rather, they help us reduce uncertainties about hepatic mechanisms enabling us to refine, explore, and test hypotheses about the mechanistic details of hepatic drug disposition. In depicting 3D hepatic morphology at a cellular level, it uses quasi-autonomous components that recognize and interact uniquely with mobile objects representing different compounds. The consequences of simulated local interactions and systemic phenomena are measured and studied simultaneously, analogous to how wet-lab experiments are conducted. Objects represent aspects of hepatic organization and function. Drugs are represented using customizable, mobile objects: more than one drug can be studied simultaneously.
When outflow profiles resulting from ISL execution are made experimentally indistinguishable from the actual profiles obtained from experiments using perfused normal or diseased livers, we can argue that the causative, mechanistic details occurring during ISL execution may have hepatic counterparts as diagrammed in Fig. 2A. Differences in mechanistic details (assembled components and how they interact with drugs) between the normal and "diseased" ISLs stand as hypotheses about differences between the normal and diseased livers. Execution of the two analogues tests that hypothesis. Having ISL profiles match referent, wet-lab profiles is evidence supporting those hypotheses. The differences in dynamic, multi-level details during execution of the two ISL types provide a plausible, physiologically based explanation of the disease-caused differences in hepatic drug disposition.

ISLs have been iteratively refined to achieve a rigorous degree of pre-specified similarity with referent outflow profiles. Starting with the same ISL used by (Yan et al., 2008a,b), we accomplished two tasks. First, using a protocol of iterative refinement, we discovered two different parameterizations of a common ISL structure. When dosed with objects representing diltiazem, ISL outflow profiles adequately matched profiles from the normal and diseased livers reported in (Hung et al., 2002a). Next, we designed and implemented methods to trace specific mechanistic events during ISL execution. We used derived measures of those events at different levels of detail to provide plausible explanations of how and why disposition differed between normal and diseased livers. We posit that aspects of those explanations have hepatological counterparts. Six ISL levels were studied: lobule, sinusoidal network, lobular zones, sinusoidal segments, cellular spaces, and intracellular contents. Traced simulation events included compounds interacting with lobular microarchitecture, cells, and intracellular factors that bind, sequester, and metabolize diltiazem. We simulated how compound may move to and through acinar spaces and provided measures of changes in local effects that may have been caused by disease. The results provide heretofore-unavailable views of how and where hepatic disposition of diltiazem and sucrose (Hung et al., 2001) may differ in normal and diseased rat livers. It provides a means of predicting plausible disposition properties in a diseased liver given a corresponding profile from a normal liver.

To date, mechanistic features of explanations encountered in the literature have not been testable:
typically, they draw on correlations and rely on static diagrams animated only by prosaic descriptions. Because the methods are extensible to whole organisms and, eventually, patients, they open a door to new experimental means of testing the plausibility of such mechanistic explanations, and that, in turn, is expected to facilitate translation of research results to benefit patients.

**Methods**

**In situ liver perfusion studies.** Full details of the original single-pass perfusion experiments are provided in (Hung et al., 2002a,b). Included are detailed descriptions of the established, carbon tetrachloride (CCl₄) treatment protocol. It was used on 150g male Wistar rats to induce acute hepatocellular injury thus providing a rat model of fibrotic, hepatic cirrhosis. Control PK profiles referred to as normal were obtained using livers from matched rats treated identically, absent CCl₄. Several histopathology measures of both CCl₄-treated and control livers characterized the nature and extent of disease. Diltiazem was one of several compounds studied. Six diltiazem outflow profiles were analyzed individually using established PK methods. The referent profile used in this study is the one presented in Fig. 1 of (Hung et al., 2002a).

**In Silico Liver and its framework.** Classical physiologically based pharmacokinetic (PK) modeling (Hung et al., 2001), (Hung et al., 2002a) and the approach used herein present different yet complementary approaches to exploring explanations of experimental data. The former provides a “black box” global description of flow, influx, efflux, binding, sequestration, and metabolism in the liver, and relates the resulting parameters conceptually to observed changes in liver pathophysiology and biochemistry. In contrast, ISLs use actual mechanisms—events occurring within precise spaces—in a manner that is more consistent with the actual intralobular arrangements, normal and pathological, than is possible with traditional mathematical models. Explanations of precisely how models of the ISL class differ from traditional physiologically based PK models are provided in the supplement to (Hunt et al., 2006). For convenience, the Appendix contains definitions for cellular automata, agent-based, and agent-
oriented models: explanations of differences are provided.

Detailed descriptions of ISL design considerations, including the mappings between ISL components and mechanisms and liver histology and physiology are available in (Hunt et al., 2006), (Yan et al., 2008a). An abridged description follows. Additional detail is provided under Results. Because the relationship between ISL spaces, components, mechanisms, and phenomena—mappings A, B, and C in Fig. 2A—can be made increasingly realistic and similar to hepatic counterparts, we use analogue when referring to ISLs and their components to emphasize that they are fundamentally different from traditional PK models. A traditional PK model is a conceptual generalization. Because our knowledge of hepatology is far from complete, neither the model nor its parameters can be made realistic and similar to hepatic features (Rescigno, 2001). Development and validation of models of the ISL class has just begun. Consequently, we are cautious in making claims about how faithfully ISL mechanisms represent actual normal or diseased mechanisms (mapping C in Fig. 2A). At this stage, the mechanistic details presented are only plausible approximations of what actually occurred. To avoid conflating (in our own minds) ISL details with the biology, and to clearly distinguish in silico components and processes from corresponding hepatic structures and processes, hereafter we use SMALL CAPS when referring to the ISL counterparts.

The ISL is a simulation framework: an in silico counterpart to an entire wet-lab experimental system (analytical instrumentation and all). It comprises an experiment agent (defined in the Appendix), which is a highly abstract analogue of the scientists conducting liver perfusion experiments, a data management module, a statistical observer module that is used to analyze data, a parameter manager, and three different liver models: DatModel, RefModel, and ArtModel. DatModel represents referent data from in situ perfusion experiments (Hung et al., 2002a). RefModel is a parameterized, classical PK model, the extended convection-dispersion model (Roberts and Anissimov, 1999), which was previously fit to referent outflow profiles. ArtModel is the complete system illustrated in Fig. 1. Because two ArtModel variants (NORMAL and DISEASED) are this paper’s focus, hereafter one Monte Carlo variant of ArtModel is called a LOBULE. We assumed that anatomical, physiological, and PK characteristics of all normal hepatic lobules are similar (Hunt et al., 2006). The LIVER is thus a collection of similar LOBULES. To
represent a single referent outflow profile, we pool results from ISL executions of 48 Monte Carlo variants of a single, parameterized LOBULE; those pooled, averaged results comprise one in silico experiment and represent a LIVER. ISL and LIVER are used interchangeably.

Each LOBULE is an abstraction: it is not intended to be an accurate, precise description of hepatic physiology nor is it intended to provide precise, accurate predictions. Rather, experiments on it help reduce uncertainties about hepatic mechanisms by refining, exploring, and testing hypotheses about mechanistic details during hepatic drug disposition. Software objects represent spatial aspects of hepatic organization and function. The consequences following executions are measured and studied simultaneously, analogous to how wet-lab experiments are conducted. Studies of ISL mechanisms during execution are expected to improve insight into the details of hepatic drug disposition in much the same way that study of interactions of drugs and hepatocytes in cultures can provide translatable insight into the hepatic disposition of drugs in whole organisms.

The LOBULE component. A LOBULE is a stand-alone software device. It includes components designed to mimic targeted lobular anatomical and physiological attributes believed to play roles influencing the disposition and clearance of drugs (Fig. 2). LOBULE components can be modified and plugged together in different ways as needed to represent different lobular properties (Hunt et al., 2006).

A principle guiding LOBULE and LIVER development has been that when measures of its behaviors are indistinguishable experimentally from referent wet-lab data, using some quantitative comparison (mapping A in Fig. 2A), then the ISL mechanisms and events may have hepatic counterparts (mappings B and C in Fig. 2A). The acceptability of such a mapping increases when each LOBULE component maps logically to identifiable hepatic components. Another guiding principle has been that, because any LOBULE component can be easily replaced by a more complicated component when that extra detail is needed, a LOBULE and thus a LIVER should be no more complicated than is needed to achieve the stated objective. A LOBULE has the six levels illustrated in Fig. 1. The relative arrangement of hepatic function and flow is represented at the LOBULE level using a directed graph to represent distinct arrangements of
PV to CV sinusoidal flow paths within portions of an acinus; a specific graph is called the SINUSOID network.

A network of Sinusoidal Segments. A software agent (an autonomous object that schedules its own events and interacts with other agents and objects in its environment; see Appendix for related information) called a Sinusoidal Segment (SS) represents all aspects of sinusoid function that influence drug disposition. Each SS is somewhat different and the stochastic differences are parameter controlled. A graph edge specifies a connection between two SSs. The SINUSOID network is subdivided into three zones. Zone I, being close to PV, always has more nodes; Zone III, being next to CV, has the fewest. SSs per zone were Zone I = 45, Zone II = 20, and Zone III = 5. As explained in (Hunt et al., 2006), that number was needed (Hunt et al., 2006) to have sufficient PV-to-CV path variety to reduce fluctuations within outflow profiles. They were connected using 109 edges: 39 intra-Zone I edges, 8 intra-Zone II connections (but no intra-Zone III connections), 37 Zone I-to-Zone II, and 25 Zone II-to-Zone III connections. All Zone III nodes were connected to CV. There were two constraints: no self-self edges and no two-node cycles (a restriction that was not imposed in (Hunt et al., 2006) and (Yan et al., 2008a,b)), and if any node was, by chance, not assigned an outgoing edge, it was connected directly to CV. Two SS types were used: direct (larger, shorter) and tortuous (thinner, longer).

Each SS consists of a Core surrounded by three identically-sized spaces. The Core represents blood flow. Space A represents the interface between vascular flow and the endothelial layer. Space B is called the ENDOTHELIAL layer. It represents the most easily accessible spaces and cells, primarily endothelial cells and fenestra. Space C represents less accessible spaces and cells, primarily the space of Disse, hepatocytes, and bile canaliculi. A Bile Space, illustrated in Fig. 1, can be made available when needed, but it was not needed for this study of unchanged diltiazem. Parameters allow the resolution of the spaces to be increased (or decreased) as needed. In this study, space size was the same as in (Hunt et al., 2006) and (Yan et al., 2008a,b).

CELLS are components within Sinusoidal Segments. Objects called CELLS were placed randomly
at a large (parameter-specified) fraction of Spaces B and C locations. CELLS in Space B, the ENDOTHELIAL layer, are thought to represent primarily endothelial cells and so are called ENDOTHELIAL CELLS. CELLS in Space C are thought to represent primarily hepatocytes and so are called HEPATOCYTES. Mobile objects represent compounds; each COMPOUND maps to a small portion of the dose. DILTIAZEM but not SUCROSE could enter and exit CELLS.

CELLS contain objects that represent required intracellular components and processes, including drug binding, metabolism, transport, and sequestration. Because the in situ perfusions had short durations (less than one hour), we assumed that cell biology and biochemistry were relatively constant. Consequently, those details have been abstracted away, but can be added easily when needed without having to reengineer ISLs. The only intracellular events that are important for studying the disposition kinetics of unchanged diltiazem were its binding, sequestration, and metabolism. However, disease can affect the intracellular hepatic transport kinetics (Hung et al., 2005a) and biliary clearance (Hung et al., 2005b) of other compounds, with biliary clearance being more likely when the solutes are large and polar (Roberts et al., 2002). As some of the polar diltiazem metabolites are cleared into the bile (Sugawara et al., 1988), the bile space is shown in the model (Fig. 1). Even though it is known that basic compounds are sequestered in organelles such as lysosomes and mitochondria, as well as being bound in hepatocytes (Hung et al., 2002a), (Siebert et al., 2004), motivated by parsimony, sequestration and binding were not resolved: drug molecules within cells are simply subdivided and represented as being in one of three states: free to exit (partition out), bound, or unbound and freely moving. Everything within a cell that can bind or sequester diltiazem was conflated and represented by some number of identical binding objects (hereafter, simply BINDERS). Within hepatocytes, we do not resolve binding to metabolic enzymes, such as members of the CYP450 family, and binding to or sequestration by other cell components. BINDING inside HEPATOCYTES is handled by BINDERS called ENZYMES, and their BINDING events can end with release of METABOLITE.

**In silico liver parameters.** The LIVER and its components from (Yan et al., 2008a,b) were re-used
for this study. See (Yan et al., 2008a) and the supplemental data for a full list of parameter names, descriptions, and values (see Supplemental Table S1). For convenience, several key parameters are listed in Table 1 and the specifics of others important to this report are discussed below and under Results. This class of synthetic, executable models was developed in part to explore and test hypotheses about biological mechanisms. An objective of this study has been to explore alternative mechanistic explanations and offer one that validates and so may account for the differences in outflow profiles of diltiazem administered to normal and diseased livers. The histopathology data showed that within the CCl₄-treated livers, significant cirrhotic change occurred at cellular and subcellular levels (Hung et al., 2002a,b). Microscopy evidence also showed that microvascular and microcirculation changes occurred (Gaudio et al., 1997). For simplicity, many of the details known about liver fibrosis, including the principal role played by hepatic stellate cells, for example, were not added to the list of targeted attributes.

It is tempting to assume that those visible changes must have contributed in important ways to the observed alterations in outflow profiles. Yet, we do not know the dispositional significance of such changes for specific compounds. We elected to make no inferences but seek one of the simpler sets of changes that could provide a plausible explanation. It may be possible, for example, that two different SINUSOID networks, with all cellular and subcellular details being unchanged, can provide an explanation for the differences. However, Hung et al. showed significant correlations between measures of histopathology and changed PK parameter values. We therefore elected to begin by re-using the validated NORMAL LIVER SINUSOID network, once identified, for the DISEASED LIVERS. We then looked for explanatory change at the SS level and below.

**Discovering differences between NORMAL and DISEASED LIVERS.** The approach taken followed three steps. First, we identified a parameterized LIVER that would validate against the diltiazem outflow profile from the normal liver with a Similarity Measure (SM) value, discussed below, > 0.8. Second, we identified a subset of parameters to be changed, and then sought focused changes in SS component parameterizations that would alter the outflow profile in ways consistent with the referent diseased
outflow profile. We identified a subset of parameters for change, and then tested the hypothesis that a new valuation of the subset, in combination with unchanged values of all other parameters, would yield a LIVER that could achieve the minimally acceptable SM > 0.8. When unsuccessful, we returned to step two. Third, we fine-tuned that candidate NORMAL LIVER and then the DISEASED LIVER parameter vectors with the objective of achieving SM > 0.9 (within a factor of 0.33 of the referent value, as discussed below). When unsuccessful, we returned to step one or two.

Discovering a parameterized, NORMAL LIVER that would validate followed a similar, iterative refinement process. We reported earlier (Hunt et al., 2006) that because the emergent influence of parameters on dynamic ISL attributes is highly networked, more than one parameter vector could give essentially indistinguishable outflow profiles. Although every effort was made to construct a minimal model, the requirement that LOBULAR structures be derived or inferred from published hepatic knowledge rather than induced from particular data sets provides some complexity and overlapping phenomena. Such complexity, natural to constructive models, can be misinterpreted as “over fitting.” ISLs were not over fitted because a) they were not actually fitted at all: the values of the parameters after tuning are not thought to reflect some general physiological truth, and b) their complexity better reflected the situational adaptability of the referent liver. It is therefore not surprising that we could identify a parameterized NORMAL LIVER that validated, yet no straightforward strategy was found to successfully transform it, following the above three-step protocol, to yield a DISEASED LIVER that also validates.

**Drug input and dosage time management.** ISL experiments followed the same protocol used by (Hung et al., 2001) and (Cheung et al., 1996). Sucrose was often co-administered with the drug of interest as an extravascular marker. Abnormalities in its outflow profile can indicate pathologies that otherwise might not be evident. We did the same. A bolus dose divided equally between SUCROSE and DILTIAZEM was injected into a simulated catheter that feeds into PV. COMPOUNDS were collected as they entered CV, simulating being collected by a fraction collector. Hung et al. used the sum of two inverse Gaussian density functions (requiring five-parameters) with a lag-time to simulate compound dilution and
dispersion within catheters and perfusion tubing (Hung et al., 2001), (Hung et al., 2002a,b). They fitted
the density function to averaged outflow profiles obtained following their standard experimental protocol
when the liver was replaced by a shunt. They then used that fitted dosing function to correct outflow
profiles prior to PK analysis. We obtained identically-shaped dosing curves using the three-parameter
density function $d(t)$. As done earlier (Hunt et al., 2006), (Yan et al., 2008a,b,c), we parameterized $d(t)$ to
provide quantitative control of COMPOUND input into PV and to simulate all influences on diltiazem in
situ prior to reaching PV and after it exiting CV, before actual collection. Parameters $a$, $b$, and $c$
determine the amplitude, location, and shape of the input density function; $t$ is simulation time (TIME).

$$d(t) = a \cdot \frac{b^c \cdot t^{c-1} \cdot e^{-b \cdot t}}{(c-1)!}$$  \hspace{1cm} (1)

Inspection of the referent outflow profiles in Fig. 3 showed that diltiazem from the cirrhotic liver
appeared sooner in the collected effluent, and its outflow profile peak occurred sooner. Because the
catheters and perfusion apparatus were the same, we interpreted these changes as reflecting changes in
large vein effects combined with differences in the more rapidly equilibrating, immediately accessible
fluids, factors that were represented by the dosage function. Differences in values of dosage function
parameters $b$ and $c$ (but not $a$) were explored as part of the iterative refinement process. It became clear
that using different values of $b$ would mimic the differences in early outflow values and thus facilitate
validation: for NORMAL LIVERS, $b = 1$ and for DISEASED LIVERS, $b = 2$. We used $a = 5,000$ and $c = 2$
for both LIVER types. The resulting dose input densities are shown in Fig. 3. With those values, the total
number of COMPOUNDS for each execution was 9,192 for the NORMAL and 7,232 for the DISEASED
LIVERS, divided equally between SUCROSE and DILTIAZEM. The parameters of the dosage function were
originally designed to abstractly represent only the dosing apparatus for the in situ experiment. The fact
that modifying that mechanism helps in generating profiles similar to the wet-lab diseased livers, even
though there were no changes to the experimental apparatus, demonstrates how a more coarse mechanism
like the dosage function can be refined. Wet-lab experiments targeting this refined mechanistic
hypothesis are merited.

The highest-level agent is the Experiment Agent, *ExperAgent*. One complete cycle of its actions corresponds to one simulation cycle and, as in the earlier validation studies, one *ExperAgent* simulation cycle mapped to 0.5 seconds of referent wet-lab time. There are two types of actions: macro, such as flow and relocation of COMPOUNDS, and micro, such a chemical event like BINDING. Only the SS has macro actions. Both the macro and micro actions of the *artModel* are executed twice per *ExperAgent* cycle. Only the *artModel* agent distinguishes between macro and micro actions. A micro cycle is called a step to distinguish it from a simulation cycle; each step maps to 0.25 seconds of wet-lab time. The *artModel* schedules macro actions before micro actions. Consequently, a bound DILTIAZEM can be released in one step; it can then re-bind in the next step before it is given an opportunity to move out of the CELL. All tracing algorithms operated each step to trace both micro and macro actions.

**Iterative LOBULE refinement achieved validation of LIVER outflow profiles using a quantitative Similarity Measure.** Because of inter- and intraindividual variability, having a predicted pharmacokinetic profile be within a factor of two of the actual values has often been deemed acceptable (Fagerholm, 2007). Our target criterion was more stringent: $\geq 90\%$ of simulated values should be within a factor of 0.33 of the referent values. We calculated expected referent values (between actual measured values) using the best-fit *RefModel*. Details of SM calculations are provided in the supplemental data. We calculated SM values for both raw and smoothed outflow profiles. The latter were preferred for visualization. One dimensional wavelet transform with the smoothing window size of three was used for graphical presentation of outflow data.

**Multi-scale tracing.** The spatiotemporal activities of COMPOUNDS and their interactions with LOBULAR components were traced across all five levels (Fig. 1). Tracing was divided into two phases: generating raw tracing data and then constructing derived measures of that data. During the former, all specified events involving COMPOUNDS and their interactions with LOBULAR components in their temporal order were stored. During the second phase, tracing results were constructed by applying a set
of quantitative measures. A variety of measures was specified to gain insight into how the mechanisms of COMPOUND disposition within NORMAL and DISEASED LIVERS differed. Included were Resident TIME (within a LOBULE), TIME of METABOLISM, cumulative METABOLIC events, Path Length, fraction of DILTIAZEM within the LOBULE that was bound per cycle in either CELL type, and encountered hepatic components. For the latter category, four different events were measured for each DILTIAZEM: 1) number of UNBINDING events within ENDOTHELIAL CELLS and 2) within HEPATOCYTES, 3) number of BINDING events within ENDOTHELIAL CELLS and 4) within HEPATOCYTES. We measured these processes within ISLs, but they cannot be measured in perfused livers. Nevertheless, as illustrated in Fig. 2, the evidence presented in the next section supports the hypothesis that corresponding, quantitatively similar events may have occurred during in situ perfusions.

Resident TIME measured either when a COMPOUND exited a LOBULE unchanged or was METABOLIZED. Some COMPOUNDS remained in the LOBULE when the simulation terminated. TIME of METABOLISM recorded when a bound DILTIAZEM was METABOLIZED. Path Length measured the minimum length of the path taken by each COMPOUND between PV and CV, or between PV and a specific SS upon terminating the simulation. We recorded each SS visited by a COMPOUND until it reached the CV and was collected, was METABOLIZED, or the simulation ended. We then summed the length of each SS visited. Length is measured in grid spaces. If a SS had a circumference of 24 and a length 10 grid spaces, then the minimum path length for a COMPOUND traversing that SS would be 10 grid spaces. If a COMPOUND entered and exited five such SS, then its path length would have been 50 (grid spaces), regardless of where the COMPOUND may have traveled within each SS or how long it remained in any one SS. If the COMPOUND entered a SS prior to simulation termination, but did not have an opportunity to exit, its recorded Path Length would include the length of that SS.

Multi-scale traceability data facilitated unraveling the mechanistic differences regarding where and how NORMAL and DISEASED LIVERS interacted differently with COMPOUNDS and thus the causes underlying differences in their outflow profiles. It is infeasible to obtain comparable wet-lab data. Nevertheless, we maintain that the relative tracing data has provided the current best approximation of
corresponding differences that existed within control and CCl₄-treated livers. Because the outflow profiles from NORMAL and DISEASED LIVERS validated against wet-lab counterparts, we can state the following. If we accept that a reasonably direct mapping exists between mechanisms and causes determining DILTIAZEM disposition in NORMAL LIVERS (mappings B and C in Fig. 2A) and corresponding mechanisms and causes in normal perfused livers, then we can hypothesize that the mechanisms and causes determining DILTIAZEM disposition in DISEASED LIVERS are a reasonable approximation of corresponding mechanisms and causes in CCl₄-treated perfused livers.

**ISL implementation and execution.** NORMAL and DISEASED LIVERS were designed using multi-agent based modeling and simulation methods (Hunt et al., 2006). Swarm 2.2 (www.swarm.org) and GCC (gcc.gnu.org) 4.1.1 were used for implementation. For efficient execution of large-scale ISL experiments, all in silico experiments were performed within a distributed and parallel computing environment implemented using OSCAR (oscar.openclustergroup.org) 5.0 and MPICH (www-unix.mcs.anl.gov/mpi/mpich1/) 1.2. R 2.5.1 (www.r-project.org) and Matlab (www.mathworks.com) 7.14 were used to create and analyze tracing results.

**Results**

All results are from experiments on various ISLs. Assume, for the levels of abstraction used, that mappings A, B, and C in Fig. 2 are all realistic and accurate for the livers used in the cited wet-lab experiments. Also, assume that quantitative mappings from ISL results to wet-lab results have been shown to be acceptably precise. Under those conditions, the results of a modified ISL experiment would stand as predictions of expected results from a similarly modified wet-lab experiment, where example modifications to the experiment include using somewhat different conditions or using a different compound. Achieving that level of validation and trust in ISLs is a goal. The validation results presented below move us in that direction, but more is needed to get there. Consequently, the parameter values, results, and descriptions of change from NORMAL to DISEASED states reported below should not yet be
viewed as predictions. We present them as plausible explanations of phenomena and as plausible
descriptions of change. To emphasize that we are early on the path to strong validation and trust, we
confine our rhetoric to describing and contrasting what occurred during ISL executions, and draw
attention when those phenomena were consistent (or not) with reported wet-lab observations.

Changes in sinusoid properties at three lobular levels account for cirrhosis-caused differences
in disposition. We discovered two different parameterizations (Table 1) of a common LOBULAR
structure, one NORMAL and the other called DISEASED, which gave DILTIAZEM outflow profiles that
validated against referent outflow profiles based on quantitative Similarity Measures. Example outflow
profiles are graphed in Fig. 3. Progressive cirrhosis (caused by chronic CCl₄ treatment) iteratively
changed normal livers into diseased livers (Hung et al., 2001), (Gaudio et al., 1997). Our goal was to
discover one plausible way in which spatiotemporal interactions between DILTIAZEM and LOBULAR
components within validated NORMAL LIVERS could be changed so that the new outflow profile would
validate against corresponding data from the CCl₄-treated liver. The relative differences between the two
differently parameterized LIVERS (NORMAL and DISEASED) could then stand as a hypothesis about the
differences between the referent livers from the perspective of diltiazem (and sucrose) disposition.

Following the iterative refinement protocol, we first identified the NORMAL parameterization in
Table 1. The perfusion experiment using DILTIAZEM and SUCROSE administered together to that LIVER
gave the DILTIAZEM outflow profile in Fig. 3A. It achieved our most stringent Similarity Measure (SM)
(> 90% of simulated values are within a factor of 0.33 of referent values). We then strove to achieve the
same level of validation for DISEASED LIVERS by changing only a small subset of NORMAL LIVER
parameter values. PERFUSATE flow through each SS and the extent of local turbulence were controlled by
two parameters: CoreFlowRate and SinusoidTurbo. For simplicity, their values were set to be the same
for both LIVERS.

We focused first on tuning seven of the nine parameters (Table 1) known to be sensitive to changes
in referent drug physicochemical properties (PCPs) (Yan et al., 2008b,c). Because DILTIAZEM and
SUCROSE were used in both LIVERS, ISL2WetLabScaling and MetabolizeProb were held constant. To achieve the profile in Fig. 3B we ultimately changed eleven parameters. We did not choose a direction for parameter change based on preconceived ideas of how cirrhosis should be represented in a DISEASED LIVER. Rather, we allowed the iterative refinement protocol to guide parameter tuning. We were unable to achieve the most stringent SM for DISEASED LIVERS by tuning fewer parameters. Changes in those nine parameter values alone allowed us to achieve only the less stringent SM. We achieved the more stringent SMs by also changing slightly the mix of SS types used (SSTypeRatio) and the densities of CELLS (HEPATOCYTES) in Space C. In addition, SS circumference in all NORMAL LIVERS was tuned to 24 (grid spaces), whereas in the DISEASED LIVERS it was 25. That increase is consistent with the reported increased size of the Space of Disse in diseased livers (Gaudio et al., 1997). SSTypeRatio (Table 1; Fig. 4i) controlled the ratio of direct (larger, shorter) and tortuous (thinner, longer) SS. NORMAL LIVERS used 95% direct, whereas DISEASED LIVERS used 99%. For each Monte Carlo LOBULE variant, each SS length was determined pseudo randomly to reflect uncertainties and biological variability. A set of five parameters specified their dimensional limits. Lengths for each SS were drawn randomly from a modified Gamma distribution (Hunt et al., 2006).

The densities of CELLS in the ENDOTHELIAL and HEPATOCYTE layers (Spaces B and C) were controlled by separate parameters: ECDensity and HepDensity, respectively. The final, tuned value of ECDensity (0.65) was the same for the two LIVER types, whereas HepDensity was tuned to 0.70 in NORMAL and 0.65 in DISEASED LIVERS (Fig. 4E). That corresponds to the observed, CCl4-induced rearrangement of hepatocyte quantitative ultrastructure (Hung et al., 2002b).

It was essential to change all four of the parameters that control COMPOUND movement between Spaces A, B, and C (Fig. 4A–C, H): A2BJumpProb, B2CJumpProb, B2AJumpProb, and C2BJumpProb. Lower values for the first two mean that DISEASE reduced access of COMPOUNDS to ENDOTHELIAL CELLS and HEPATOCYTES, which is consistent with fibrotic changes (Hung et al., 2002a). The effective ability of Space C to retain COMPOUNDS (B2CJumpProb/C2BJumpProb) in a DISEASED LOBULE (0.69) was smaller than in a NORMAL LOBULE (1.25), and that is consistent with disease-caused changes in hepatocyte
ultrastructure (Gaudio et al., 1997). However, the effective capacity of Space B ([A2B\text{jumpProb} + C2B\text{jumpProb}] / [B2C\text{JumpProb} + B2A\text{jumpProb}]) in a DISEASED LOBULE (1.12) was larger than in a NORMAL LOBULE (0.93), which is consistent with observed fibrotic changes (Hung et al., 2002a).

The number of BINDERS per CELL (Fig. 4D) needed to validate the DISEASED LIVER, relative to NORMAL, dropped from 95 to 65. BINDER properties also changed. In DISEASED LIVERS, relative to NORMAL, the probability of a BINDING event (Fig. 4F) occurring within a step (0.25 SECONDS) decreased from 0.5 to 0.35, whereas the binding duration (Fig. 4I) increased considerably from 11 to 28 steps (2.75 to 7 SECONDS), as if the reported disease-caused changes in the biochemical nature of the cellular interiors in cirrhotic livers (Hung et al., 2002b) favored prolonged diltiazem sequestration. Acceptable SM values were obtained without having to change the probability that the COMPOUND released from an ENZYME (in HEPATOCYTES) would be a METABOLITE: \( \text{MetabolizeProb} = 0.02 \) for both NORMAL and DISEASED LIVERS.

The combined effects of the above differences changed the temporal location of DILTIAZEM within DISEASED relative to NORMAL LIVERS. For example, in DISEASED LIVERS a larger fraction was retained in HEPATOCYTES, relative to ENDOTHELIAL CELLS, yet fewer METABOLITES were produced.

**Simulated DISEASE changed how COMPOUNDS moved through LIVERS and where they spent TIME.** Within the 100 SECOND simulation interval, 73.9% of DILTIAZEM administered to NORMAL LIVERS was collected unchanged at the CV or was METABOLIZED. The corresponding value for DISEASED LIVERS was 67.5%. The spatiotemporal history of each COMPOUND moving through each of the 48 LOBULES was recorded. DILTIAZEMS were traced using all of the measures described under Methods. However, SUCROSE was traced using only Resident TIME and Path Length. Because SUCROSE did not enter CELLS, there were no intracellular tracing results. Resident TIME within a LOBULE measured either when a COMPOUND exited a LOBULE unchanged or was METABOLIZED. Resident TIMES are graphed in Fig. 5A–D. Most striking were the different graph shapes. Comparing the first bar (Fig. 5A, B), for example, we see that the fraction of DOSE having Resident TIMES \( \leq 10 \) SECONDS was reduced in the DISEASED LOBULE by a factor of 3.2. That change is consistent with known vasculature changes combined with increased
fibrosis.

The differences in Resident TIMES (Fig. 5A-D) reflect the combined effects of all changes. To illustrate, because parameter \( A2BJumpProb \) (NORMAL/DISEASED) = 0.38/0.21, fewer DILTIAZEMS gained early access to DISEASED Space B, relative to NORMAL Space B. Similarly, because of differences in \( B2CJumpProb \) (NORMAL/DISEASED = 0.55/0.38), DILTIAZEMS that made it to DISEASED Space B were retarded in accessing Space C. The larger dose fraction (by a factor of 1.5) in the last bar for the NORMAL relative to DISEASED LOBULE (Fig. 5A, B) was a consequence of more DILTIAZEMS having reached the HEPATOCYTE layer combined with BINDING to ENZYMES and the fact that the effective capacity of the HEPATOCYTE layer (\( B2CJumpProb/C2BJumpProb \)) in a NORMAL LOBULE (1.25) was larger than in the DISEASED LOBULE (0.69). These same mechanistic trends are even more striking for SUCROSE because it was confined to EXTRACELLULAR spaces (Fig. 5C, D): the combined effects of the DISEASED-altered parameterizations caused much more SUCROSE to exit sooner and prevented more SUCROSE from gaining access to spaces distant from the SS Cores; the latter is evident because the DISEASED LOBULE exhibited no SUCROSE Resident TIMES between 80 and 100 SECONDS. Examples of tracing the actual paths taken by specific DILTIAZEMS are provided in the supplemental data (see Supplemental Fig. S1).

**DISEASE-reduced access to ENZYMES contributed to reduced METABOLISM.** DILTIAZEMS that reside longer in a LOBULE have a higher likelihood of being METABOLIZED if access to ENZYMES is unchanged. In Fig. 5E and F we see that, qualitatively, the pattern of METABOLIC event occurrence has a TIME-shifted relationship with Resident TIMES patterns for both NORMAL and DISEASED LOBULES. The cumulative number of METABOLIC events (Fig. 5E, F) shows significant reduction in DILTIAZEM METABOLISM, even though \( MetabolizeProb \) (which maps to intrinsic clearance) = 0.02 for both NORMAL and DISEASED LOBULES. The following were five contributing factors. 1) The likelihood of DILTIAZEM being in the HEPATOCYTE layer in the DISEASED LOBULE was lower because \( C2BJumpProb \) (Fig. 4H) was larger (0.55 vs. 0.48 for NORMAL) and 2) \( B2CJumpProb \) (Fig. 4B) was smaller (0.38 vs. 0.55 for NORMAL) in the DISEASED LOBULE. 3) ENZYMES (BINDERS) per CELL were reduced from 95 to 65 (Fig.
4D) in DISEASED HEPATOCYTES.  4) For an unbound DILTIAZEM in a DISEASED HEPATOCYTE, the probability of binding to an ENZYME during any step was reduced from 0.5 to 0.35 (Fig. 4F).

5) *Solute Binding Cycles* (Fig. 4I) for ENZYMES in DISEASED HEPATOCYTES was increased from 2.75 to 7 SECONDS.

**Longer Path Lengths were absent in DISEASED LIVERS and the spatiotemporal pattern of BINDING changed.** The Path Lengths in Fig. 6A–D measured the cumulative length of all SSs entered by each COMPOUND. NORMAL and DISEASED LIVERS had 70 SS nodes distributed among the three zones. On average, NORMAL LIVERS had 4.5 of the long, narrow SS, whereas only occasionally did a DISEASED LIVER have even one. Consequently, a small subset of both DILTIAZEM and SUCROSE Path Lengths in NORMAL LIVERS were long (second peak in Fig. 6A, C), but they were essentially absent in the DISEASED LIVERS. The long, narrow SS, in combination with intra-zone edges represent the interconnections between sinusoids that are most numerous in the periportal region, yet are absent in the perivenous region of normal lobules. Microscopy evidence suggests that some of those interconnections are lost in cirrhotic lobules (Gaudio et al., 1997).

Despite differences in the parameter values that influence BINDING (Table 1: *BinderPerCell*, *Solute Binding Prob*, and *Solute Binding Cycles*), the data in Fig. 6E and F show that the fraction of COMPOUNDS that was in the LOBULE at a particular TIME and was attached to a BINDER eventually reached a similar steady state ratio of about 0.8 in both NORMAL and DISEASED LOBULES. However, the relative fractions BOUND in ENDOTHELIAL and HEPATOCYTE layers were different.

Everything within hepatocytes capable of binding or sequestering diltiazem was conflated and represented using one BINDER type. Only a small subset of that material includes enzymes that metabolize diltiazem. Because of how events were scheduled, it is possible for a DILTIAZEM to be released toward the end of one simulation step and—by chance—be bound again to another BINDER in that same CELL before being given an opportunity to move out of the CELL.

**DISEASE increased the TIME spent by DILTIAZEM within CELLS and changed the amount of...**
**TIME spent bound.** The differences in Resident Times, METABOLIC events, and Path Lengths traced in large part to the specific consequences of components encountered by DILTIAZEM at the lower levels (Fig. 1). Four pairs of encounter records are presented as bar graphs in Fig. 7. There was no corresponding SUCROSE data because SUCROSE did not enter CELLS. A record was kept of each DILTIAZEM location at each TIME step. The DOSE fraction that experienced an event (regardless of order) is indicated by bar height. Reflection on the similarities and differences for NORMAL and DISEASED LOBULES provides deeper insight into causes underlying features of the LIVER outflow profiles in Fig. 3. For example, Fig. 7C shows that in a NORMAL LOBULE approximately 12.6% of the DOSE spent 2-to-3 SECONDS unbound in HEPATOCYTES before exiting the LOBULE, being METABOLIZED, or having the simulation terminate, whereas in a DISEASED LOBULE (Fig. 7D) the corresponding value was 19.2%. That may seem counterintuitive initially, because there was more METABOLISM in NORMAL LIVERS. However, in DISEASED HEPATOCYTES, fewer ENZYME release events yielded a METABOLITE. Interestingly, the pattern was reversed for DILTIAZEMS spending 6-to-7 SECONDS in HEPATOCYTES. To demonstrate the nonlinearities of changes within SSs, the specific detailed changes for SS node #27 are provided in the supplemental data (see Supplemental Fig. S2).

To achieve the DISEASED LIVER validation in Fig. 3, fewer BINDERS per DISEASED CELL (67 vs 97: Fig. 4D) were needed; in addition, the probability that a DILTIAZEM-BINDER encounter would result in a BIND event was reduced (Fig. 4F: 0.37 in DISEASED vs 0.7 in NORMAL ENDOTHELIAL CELLS and HEPATOCYTES), yet for DISEASED CELLS the effective duration of BINDING (Fig. 4I) increased by a factor of 2.77. The bar graphs in Fig. 7E and F show that these differences gave rise to very different BINDING records for DILTIAZEM in NORMAL and DISEASED ENDOTHELIAL CELLS. Almost 70% more DILTIAZEMS spent 0–8 SECONDS bound within NORMAL as compared to DISEASED ENDOTHELIAL CELLS. On the other hand, a small percentage of DILTIAZEM spent over 40 SECONDS bound within DISEASED ENDOTHELIAL CELLS (as if the DILTIAZEM were entangled by the simulated effects of fibrosis), whereas none spent 40 SECONDS or more bound within NORMAL ENDOTHELIAL CELLS.
The consequences of small parameter changes may not be evident. How sensitive are the characteristics of an outflow profile to changes in values of the key parameters in Fig. 4? Outflow profile sensitivity to parameter changes is discussed in (Hunt et al., 2006): because all ISL mechanisms are networked, a change in ISL phenomena produced by a change in one parameter can often be offset by smaller, compensating changes in the values of several other parameters. Consequently, studies of sensitivity to individual parameters are less informative than are location changes in LOBULE parameter space. Individually, the parameter changes in Fig. 4 did not cause statistically distinguishable changes in outflow profiles. Nevertheless, someone experienced in observing different ISL outflow profiles may observe a perceptible change in outflow profile shape. In general, a 5% change in any one parameter will produce an imperceptible change in an outflow profile and no change in SM value. However, a 5% change in all parameters can cause a significant change in outflow profile. The changes in Fig. 4 averaged 38.5%.

Discussion

For the level of abstraction used, the two outflow profiles from NORMAL and DISEASED LIVERS matched referent profiles (Fig. 3) reasonably well. That evidence supports the idea that both mechanism and event level mappings in Fig. 2A may be reasonable. With additional rounds of iterative refinement and validation, ISLs may provide useful scientific predictions, as well as deeper insight into causal mechanisms within multiple levels. Similarity was achieved without having to use different SINUSOID networks for NORMAL versus DISEASED LIVERS. However, it is premature to assign biological significance to that lack of difference.

All NORMAL→DISEASED LIVER changes evolved without bias as a natural consequence of the iterative tuning protocol, yet all were consistent with cirrhosis-caused changes in the local environments encountered by diltiazem and sucrose. Taken together, the NORMAL→DISEASED changes retarded DILTIAZEM and SUCROSE movement between spaces, changing the Resident Time patterns (Fig. 5) and Path Lengths (Fig. 6). The coupling of those changes with altered BINDER and ENZYME attributes (Fig. 7)
changed the time course of measures of both BINDING (Fig. 6) and METABOLISM (Fig. 5).

The cited prior results supported component-to-component mappings between NORMAL LIVERS and normal livers (Mapping C in Fig. 2A). Those similarities are reinforced by similarity of outflow profiles (Fig. 3). The fact that drug PCPs can guide specification of PCP-sensitive NORMAL LIVER parameter values that validate for additional COMPOUNDS (Yan et al., 2008b,c) strengthens the hypothesis that Mapping B exists between NORMAL LIVERS and their normal counterparts. Similar evidence is not yet available for DISEASED LIVERS. Nevertheless, we suggest that the incremental parameter changes that are necessary (and sufficient) to transform a NORMAL into a DISEASED LIVER (Fig. 2B) may correspond abstractly to molecular, cellular, and sinusoid level transformations responsible for the pathogenesis from normal into diseased livers during CCl₄-treatment. The general consistency noted in Results between DISEASED LIVERS during execution and the cited histopathology evidence supports the hypothesis. We thus have a tentative, yet promising, in silico model that enables us to visualize, abstractly from the perspective of diltiazem, how the consequences of cirrhosis may have progressed. That new capability represents an important step toward unraveling the complex influences of disease on drug disposition illustrated in Fig. 2B. Being able to transform one validated model into another is also important: it is evidence that the approach can facilitate rational translation of research results to useful applications (“bench to bedside”), and that may open doors to development of strategies for tailoring drug choices to help reverse disease conditions.

When needed, additional objects can be added easily to Space C and HEPATOCYTES to enable extending ISL mechanisms to solutes or metabolites that are actively transported across the basolateral membrane, into the cell, or across the canalicular membrane to bile. So doing would enable exploring disease-caused changes in transporter expression. We would start with TRANSPORTER agents that have been used in other models of this class. To date TRANSPORTER agents have been validated and used within analogues of cell cultures used in transport studies (Garmire et al., 2007), (Lam and Hunt, 2008), including hepatocytes (Sheikh-Bahae S and Hunt, 2006), and an in an analogue of the small intestine (Garmire and Hunt, 2008).
Our strategy in developing and evolving synthetic analogues has been to iteratively expand the biologically relevant phenotype of the analogue so that it has an increasing variety of measurable attributes in common with referent systems. By so doing, we increase the variety of ways Mappings B and C in Fig. 2A can be concretized. For this study, there were four items on the targeted attribute list. First, outflow profiles of DILTIAZEM from NORMAL LIVERS must also be similar (using a stringent SM) to the referent outflow profile. Second, outflow profiles of DILTIAZEM from DISEASED LIVERS must also be similar (using the same stringent SM) to the diltiazem outflow profile from a diseased liver. Third, the DISEASED LIVERS are achieved by simple transformation of NORMAL LIVER components and their properties. Finally, that the NORMAL-to-DISEASED changes are consistent with histopathology data. To build confidence in Mappings B and C and the additional relationships illustrated in Fig. 2B, the set of targeted attributes for validation must be expanded. To achieve that objective, new wet-lab experiments will be helpful, but may not be essential: we can draw further on the abundance of relevant histopathology evidence available in the literature. For example, we could add two evidence-based attributes: that fibrosis retards access of small molecules to hepatocytes, and in cirrhotic livers, there are fewer fenestrae. For those additions, the current DISEASED LIVERS would still validate because tuned values of both A2BJumpProb and B2CJumpProb in Fig. 3 are lower for the DISEASED LIVERS. Biologically, those lower values correspond to (predict) two differences between CCl₄-treated relative to normal livers. 1) The probability of compounds like diltiazem entering the endothelial layer is reduced. 2) A compound within the endothelial layer will be retarded in passing through to the hepatocyte layer. For some other attributes, however, their inclusion in the list of targeted attributes would automatically falsify the current DISEASED LIVER. An example might be that the frequency of periportal interconnections between sinusoids is reduced in diseased livers. With that addition, we would need to seek a valid parameterization of a new DISEASED LIVER in which the SINUSOID network differs from the current one in measurable ways. With such future uses in mind, the ISL has been designed purposefully (Hunt et al., 2006) to make such change relatively easy.
Being able to explore and easily implement various derived measures such as those in Figs. 5–7, opens the door to future rounds of revision and validation that draw on the available variety of accumulated experimental evidence, whether or not drug disposition was a consideration in the design of the original wet-lab experiments. Such efforts would strive to draw on both biological and simulation insights gained from related modeling efforts, such as the individual cell-based, off-lattice mathematical model developed by Höhme et al. of liver regeneration at the lobule level after acute CCl₄ injury (Höhme et al., 2007).

Having independently validated NORMAL and DISEASED LIVERS allows one to explore plausible drug disposition consequences of intermediate levels of disease and even disease that is more advanced. Because of individual differences in disease progression, conducting wet-lab experiments to document the former would be problematic, and the latter may be deemed unethical. By assuming disease progression corresponds to gradual change from NORMAL to DISEASED LIVER parameter values, we can simulate a liver that has progressed half way, for example, along the path to the currently documented disease state. We can project further parameter changes to explore plausible consequences of more advanced level of disease. Corresponding explorations of intermediate and advanced disease states would be infeasible using traditional inductive mathematical models.

The methods and approach have been designed to enable the eventual development of horizontally and vertically integrated whole organism—whole patient—analogues. Once that goal has been achieved, it will be feasible to use in silico experimentation to anticipate PK properties of drugs in patients with liver disease.
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Footnotes

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Legends for Figures

Figure 1. In Silico Liver. Illustrated are the six LOBULAR levels. 1) LOBULE, 2) the SINUSOID network maps to sinusoidal flow paths from terminal portal vein tracts (PV) to the central hepatic vein (CV). The SINUSOID network is divided into three zones (zonation is level 3). The validated network size and general structure emerge from iterative parameter tuning; specific structure is Monte Carlo determined, and is unique for each LOBULE. At each node within a SINUSOID network is a Sinusoidal Segment (SS). A graph edge specifies a flow connection between two SS. 4) A SS maps to aspects of sinusoidal architecture and function. Objects representing DILTIAZEM and SUCROSE enter and exit a SS via the Core and Space A; Space A represents the interface of blood flow and the endothelial layer. 5) SINUSOID spaces contain objects representing functions. Space B is called the ENDOTHELIAL layer; it represents the most easily accessible spaces and cells, primarily endothelial cells and their fenestra. The outermost Space C, called the Space of Disse and HEPATOCYTE layer (HEPATOCYTE layer for short), represents the less accessible spaces and cells, primarily the space of Disse and hepatocytes. Each space can be replaced by one or more finer-grained spaces should additional detail be needed. An additional space representing bile can be made available when needed, but for this study, it was not needed. 6) Objects called CELLS occupy a large fraction of Spaces B and C; they represent cells and function as containers for other objects. In Space B they are called ENDOTHELIAL CELLS; in Space C, HEPATOCYTES. Objects representing all needed intracellular components can be placed within CELLS. For DILTIAZEM, only two BINDING object were needed. In ENDOTHELIAL CELLS, a BINDERS simply binds and releases DILTIAZEM. In HEPATOCYTES, ENZYMES can release either unchanged DILTIAZEM or a METABOLITE. The potential for heterogeneous properties within different spaces is illustrated by the expanded portion of Space B having different shadings.

Figure 2. Illustration of relationships between ISL components and mechanisms and perfused liver counterparts. A: Illustrated are three levels of relationships between ISL mechanisms and components, and corresponding hepatic components and drug disposition mechanisms in either a normal or a diseased
liver. The ISL validates when the similarity between ISL outflow profiles and referent liver perfusion profiles (mapping A) are judged adequate. Once that has been achieved, we can state that the traced events occurring within the ISL during execution may have had hepatic counterparts that occurred during drug disposition: traced event mapping B is plausible. For the level of abstraction used, if the ISL components and their arrangement are judged acceptable representations of their hepatic counterparts at all six levels, then the detailed mechanisms causing the traced events may correspond to the hepatic mechanisms that occurred during drug disposition: mechanism mapping C is plausible. B: Upon validation for the attributes targeted, we can use intermediate parameterizations to document the incremental transformation of a NORMAL to a DISEASED LIVER. The details of such an in silico transformation provide a working, abstract hypothesis for the mechanisms of actual disease progression.

Figure 3. Hepatic and ISL outflow profiles are compared. Open circles: smoothed (three point) mean DILTIAZEM levels (fraction of dose per collection interval) from a tuned LIVER, which is comprised of 48 Monte Carlo executions of the same LOBULE tuned to the values in Table 1. Centerline within shaded band: trend line of referent diltiazem outflow fractions from perfused livers (Hung et al., 2002a). Values within the shaded band are within a factor of 0.33 of referent values. The most stringent, targeted Similarity Measure (SM) was that > 90% of simulated outflow values be within a factor of 0.33. A: Referent outflow profile is from a normal liver. The SM value is 0.936 without and 0.995 with smoothing. B: The referent outflow profile is from a CCl₄-treated liver. The SM value is 0.916 without smoothing and 0.980 with smoothing. Inserts in A and B show the tuned dose input profile from equation 3 used in each case. Values are fraction of dose collected when PV was connected to CV absent a LIVER. In both cases, dosing started at \( t = 0 \). We re-used and tuned the previously validated dose input function (Hunt et al., 2006), (Yan et al., 2008a), equation 1. Its parameterization is listed in the supplemental data (see Supplemental Table S1).

Figure 4. Parameter differences: NORMAL vs. DISEASED LIVERS. Of the parameters listed in Table 1, the changes in these nine contributed most to the differences between NORMAL and DISEASED LIVERS.
Specific examples of translating the LOBULE parameter to referent livers follow. **A:** $A2BjumpProb$: probability of moving from sinusoid blood to endothelial layer. **B:** $B2AJumpProb$: probability of moving from endothelial layer back to blood. **C:** $B2CJumpProb$: probability of moving from endothelial layer to hepatocyte layer and space of Disse. **D:** $BinderPerCell$: material within cells that bind diltiazem. **E:** $HepDensity$: relative density of hepatocytes (within the hepatocyte layer). **F:** $SoluteBindingProb$: a measure of the affinity of cellular material for diltiazem. **G:** $SSTypeRatio$: relative frequency of longer, shorter sinusoidal segments. **H:** $C2BjumpProb$: probability of moving from the space of Disse and hepatocyte layer to the endothelial layer. **I:** $SoluteBindingCycles$: diltiazem’s tendency to remain bound within cells.

**Figure 5.** Resident TIMES and METABOLIC events in a NORMAL and DISEASED LOBULE are compared. Vertical lines on alternate bars show the range of values for all 48 LOBULES. **A–D:** Resident TIME recorded how long each DILTIAZEM or SUCROSE resided in a NORMAL or DISEASED LOBULE. We traced each COMPOUND from initial injection until it was METABOLIZED, cleared from the LOBULE through the CV, or the experiment terminated. Bar heights measure the fraction of COMPOUND administered that had Resident TIMES within the indicated ten-SECOND interval. **E–F:** METABOLIC events recorded when a DILTIAZEM was METABOLIZED. Once a DILTIAZEM became bound to an ENZYME, a release event was scheduled at a future step (controlled by $SoluteBindingCycles$). At the scheduled TIME, either DILTIAZEM or a METABOLITE was released. Which event occurred was controlled by $MetabolizeProb$. The TIME of a METABOLIC event was recorded. Bar heights measure the fraction of DILTIAZEM dose METABOLIZED within the indicated ten-SECOND interval. The curves are cumulative fraction of DILTIAZEM dose that has been METABOLIZED. Values correspond to the right side axes.

**Figure 6.** A measure of the length of paths traveled and fraction bound in the ENDOTHELIAL and HEPATOCYTE layers (Spaces B and C) are compared in a NORMAL and DISEASED LOBULE. Vertical lines on bars: same as in Fig. 5. **A–D:** Path Length is the sum of grid space lengths of each SS visited by a COMPOUND until it was collected or METABOLIZED, or the simulation ended, regardless of where the
COMPOUND may have traveled within each SS or how long it remained in any one SS. If the COMPOUND entered a SS prior to simulation termination, but did not have time to exit, its recorded Path Length would include the length of that SS. Bar height measured the fraction of administered COMPOUND that experienced a Path Length within the indicated range. **E–F:** Measured was the fraction of administered COMPOUNDS bound (within the LOBULE at the indicated TIME) at each TIME after experiment execution. Top curve: total fraction bound. Bottom curve: fraction bound within HEPATOCYTES within Space C. Middle curve: fraction bound within ENDOTHELIAL CELLS within Space B.

**Figure 7.** The TIME spent by each DILTIAZEM unbound **A–D:** or bound **E–H:** within a CELL type are compared. The bar graphs show the fraction of DILTIAZEM dose spent associated with the indicated SS component, irrespective of event sequence. Vertical lines on bars: same as in Fig. 5.
Table 1. Key ISL parameters controlling disposition in normal and diseased livers

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* In this study, this parameter is sensitive to a compound’s PCPs.
Figure 6

(A) NORMAL

(D) DISEASED

(B) DILTIAZEM

(C) SUCROSE

(E) Total

(F) Total

ENDOTHelial CELLS

HEPATocytes

PATH LENGTH (grid spaces)

FRACTION IN LIOUIBLE Bound

SECONDS
Appendix

It can be useful to describe agent-based models as they relate to cellular automata. A cellular automaton (CA) is a discrete collection, usually in the form of a regular grid of “cells” (grid spaces). Each “cell” is associated with a state (e.g. On/Off or containing a number, color, or letter). “Cell” states change over time, which is also discrete, based on the states of neighboring “cells.” The type of neighborhood determines which “cells” interact. For example, consider a CA with a square grid and a neighborhood defined as North, South, East, and West. The state of the center “cell” at time t_1 is a function of the states of its four neighbors at time t_0, where that function might be defined as “the center state is On if three of the four neighbors’ states were On.” State transitions and time are controlled by a global software executive and transitions are synchronous, meaning that all “cells” are updated each cycle. Usually, all “cells” have the same transition rules, but that is not always the case. A fundamental attribute of CA is the realization of non-local complex behaviors arising from the operation of local rules.

A CA can be thought of as a simple type of object-oriented program (OOP), where objects represent the “cells” and the transition rules are the object’s methods. The only differences being that 1) the objects in an OOP can determine with which other objects they interact, 2) their interactions are not necessarily synchronous, and 3) any object may have more than a single state transition rule. In essence, an OOP can be viewed as being a more heterogeneous and dynamic type of CA.

An agent-based system (ABS) adds considerable heterogeneity over and above that of an OOP. Whereas an OOP is not necessarily synchronous, the control of when an object interacts with another object and which objects interact is still handled by a global executive. Objects are reactive slaves to this global executive, even in a parallelized OOP. Within an ABS, on the other hand, some of the executive's capabilities and responsibilities, including some or all of the scheduling of actions, are distributed—delegated—to objects called agents. An agent has its own schedule and can be quasi-autonomous. It senses and is part of its environment, which may or may not be discretized in the form of a grid. It pursues an agenda within a larger script. An agent can choose dynamically with which other agents or
objects to interact, when to engage other agents or objects, and which of various actions to take. It can also begin engaging in new actions spontaneously without being told to do so or how to do so by a global executive. Likewise, it can decide to stop engaging in a given interaction. In fact, an agent can initiate or end the execution of any of its logic, internal or interactive. Given those attributes, “agent” can be defined technically as an object within an OOP with the ability to schedule its own actions. In models such as an ISL, an agent, like an actor, plays a role, participates in a process, or acts on behalf of something else. Importantly, an agent is identifiable by an observer as a cause of an effect. Some of an agent’s attributes and actions may be designed to represent biological counterparts; others will deal with issues of software execution.

Not all objects in an ABS need to be agents. Simpler, passive objects can fill many roles. Within the ISL, for example, COMPOUNDS are simple, reactive objects, whereas each LOBULE and each SS within a LOBULE is an agent. It can be important to distinguish an ABS from an agent-oriented system. In the former, all the capabilities described exist in the software, itself. In the latter, the actual software may not have all the capabilities of an ABS, but when the system is used, it is useful to think of the software as being composed of agents. In that sense, a CA may be agent-oriented but not agent-based. However, an ABS is sufficiently far removed from a CA so that the analogy only has pedagogical value.