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The virtual laboratory approach to pharmacokinetics: design principles and concepts

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Modeling and simulation in pharmacokinetics has turned into the focus of pharmaceutical companies, driven by the emerging consensus that *in silico* predictions, combined with *in vitro* data, have the potential to significantly increase insight into pharmacokinetic processes. To support *in silico* methodology adequately, software tools need to be user-friendly and, at the same time, flexible. In brief, the software has to allow the modeling of ideas that go beyond the current knowledge – in the form of a virtual laboratory. In this review, we present and discuss the necessary design principles and concepts required to do this. They have been implemented in the software package MEDICI-PK, demonstrating its feasibility and advantages.

Pharmacokinetics in drug discovery

The medical benefits of a drug depend not only on its biological effect at the target protein but also on its life-cycle within the organism – from its absorption into the blood, distribution to tissue and its eventual breakdown or excretion by the liver and kidneys. Pharmacokinetics (PK) is the study of the drug–organism interaction, in particular the investigation of ADME–Tox processes [1,2]. Studying ADME–Tox profiles is widely used in drug discovery to understand the properties that are necessary to convert leads into good medicines [3,4].

As a result of studies in the late 1990s, indicating that poor PK and toxicity were important causes of costly late-stage failures in drug development, it has become widely appreciated that these areas should be considered as early as possible in the drug discovery process [3]. A great deal of *in vitro* data on physicochemical properties and specific ADME–Tox processes is already available at early stages of the drug discovery process. These data, related to new drug candidates, can be used in physiologically based PK (PBPK) models to predict, analyze and optimize the PK of the compounds [5,6]. For instance, a combination of only five compound-specific parameters (fraction unbound in plasma, blood:plasma ratio, intrinsic clearance, pK_a and octanol–water partition coefficient) and known species-related physiological parameters are required for the first PK estimates of an intravenous (i.v.) application [5]. Along the drug discovery process, more-detailed compound-specific data and *in vivo* data are generated, upon which more-detailed predictions and analyses can be made. The PBPK model approach is flexible, in the sense that it has the potential to be continuously updated in the light of new information, whether physiologic-, disease- or drug-related, including upregulation and downregulation of crucial components [7]. In particular, PBPK modeling has the advantage of being able to incorporate experimental animal data, as well as *in-silico*-derived and *in vitro* data, into a coherent framework, from which meaningful and reliable assessments can be made [8].

It is worth noting that, in toxicology physiologically based models have a longer history and have been successfully applied, because of the almost exclusive reliance upon animal data for the assessment of toxicological risk to humans [9–11].

In silico modeling

In the engineering sciences, computational approaches form an integral part in the development process—some biological disciplines (e.g. systems biology) have recently proceeded in the same direction [12,13]. Modeling and simulation help to analyze and understand large complex systems, in particular when the system comprises several subunits interacting in a strong and time-dependent manner. This is exactly the situation present in PK, where

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such subunits are typically physiological processes (such as transport by the blood circulation, binding to macromolecules, permeation through membranes, metabolism by the liver enzymes, excretion by the kidneys, and so on). Although each of the processes is comparably simple the overall resulting behavior is not.

There is no single established model underlying PK, and it will remain out of reach in the foreseeable future. This situation is completely different from, for example, quantum mechanics or electrodynamics, where the Schrödinger equation and the Maxwell equations are the basis and starting point for modeling and simulation attempts, respectively. Instead, the type of model needed will depend on the question to be resolved and, therefore, on the drug development stage at which the question is addressed. *In silico* approaches will continue to evolve rapidly, just as *in vitro* methods did during the past decade [8]. The quality and the predictive power of new models will be judged against experimental data as well as against other models (for example see the study in Ref. [14]). A variety of different models for oral absorption are available [15–19]. By comparing these absorption models for different test compounds, it should be possible to understand the 'domain of applicability' of each model (i.e. under which conditions and for which class of compounds the models give the most reliable predictions). Partition coefficients are another example; there are different *in silico* methods to determine partition coefficients [20,21]. It would be interesting to compare not only the different predicted partition coefficients but also *in silico* PK studies for a list of test compounds, based on different partition coefficients. This would allow for a sensitivity analysis to identify the most crucial partition coefficient. To support this type of comparative study, flexibility in modeling will be crucial.

The present status of available software tools for modeling and simulation in PK has been thoroughly summarized [7]. The software ranges from: (i) general-purpose high-level scientific software, such as Berkeley Madonna, Matlab, MLAB and Octave; (ii) biomathematical modeling software, such as ADAPT II, ModelMaker, NONMEM and WinNonlin; (iii) toxicokinetic software, for example ACSL Toxicology Toolkit and SimuSolv; and (iv) physiologically based custom-designed software, such as GastroPlus, Pathway Prism, PK-Sim and Physiolab. As stated in Ref. [7], 'it appears that there is an inverse relationship between user-friendliness and flexibility'. However, combining flexibility with user-friendliness is exactly the domain of a virtual laboratory.

The modeling situation is comparable with the situation in systems biology [12,13,22,23], where a large variety of modeling and simulation tools are available. Software tools like COPASI, E-CELL and Virtual Cell, and many more, have been designed to fulfill the needs of systems biology modeling (a comprehensive list of software in this field can be found online; http://sbml.org). These tools are modular and open and allow for the implementation of arbitrary models. Sometimes, typical reaction schemes are pre-implemented (such as linear or Michaelis–Menten kinetics), but flexibility is almost always retained. In principle, software tools developed in systems biology could be used in PK as well; however, they do not support the special structure and needs in PK. Jointly with the upcoming modeling efforts, there has been a collective initiative of several research institutions to standardize models to facilitate exchange of models between different tools. As a result, the systems biology markup language (SBML) was created, a computer-readable format for representing models of biochemical reaction networks [24]. SBML is applicable to metabolic networks, cell-signaling pathways and regulatory networks, and might also become interesting for PK.

In the light of our experience, we see the current status of modeling in PK comparable with the status of fields such as polymer chemistry ~10–15 years ago. Since then, experts in these fields have experienced that, even without definite a priori knowledge of detailed models and parameters, the ongoing modeling process reveals such a tremendous insight into the system that the modeling research usually pays off after a short time. At the same time, significant feedback between modeling progress and experimental methods could be observed. Sometimes new modeling ideas required better or newly designed experiments; sometimes a breakthrough of analytical techniques enforced more-detailed modeling. We are convinced that this progress will take place in PK, in a similar way, in the near future. The development of the design principles and concepts of a virtual laboratory for PK has already shown that modeling principles from polymer chemistry, particle technology, catalysis and reaction engineering can be applied here in a structural way. After all, from a mathematical point of view, a PK model is not so different to a network of multiple-phase bioreactors.

The vision and the benefit

It is the emerging consensus that *in silico* predictions are no less predictive of what occurs *in vivo* than *in vitro* tests are, with the decisive advantage that much less investment in technology, resources and time is needed [8,25]. We believe that the combination of *in vitro* experiments and *in silico* modeling will dramatically increase the insight and knowledge regarding the relevant physiological and pharmacological processes in drug discovery. In the future, rather than performing separate target validation, biomarker identification, lead generation and optimization, candidate selection and preclinical development studies, these separate steps will be vertically integrated, accompanied by an *in silico* modeling process that brings together the knowledge gained from each of these steps into a disease-specific whole-body model. The physiologically based modeling approach offers a scientifically defensible method (instead of just an educated guess) for the integration of these various pieces of information from *in vitro* studies and other preclinical information – to evaluate the outcome under various assumptions [8]. Each pharmaceutical company can build their own model tailored to their individual indications, knowledge and experience. Rather than modeling, analyzing and measuring plasma- or tissue-drug-concentrations, these disease-specific whole-body models allow the study of effect-specific processes. The modeling process will benefit greatly from the systems biology community and their experience, insight and available models on metabolic pathways, gene-regulatory networks and signaling pathways [12,22–24,26]. The most feasible application of systems biology research is to create a detailed model of cell regulation [13], focused on particular signal-transduction cascades and molecules to provide system-level insights into mechanism-based drug discovery (for example, see Ref. [27]). Such models could help to identify feedback mechanisms that

TABLE 1

Important feature of the virtual laboratory MEDICI-PK

Property	Description	Realization in MEDICI-PK
User-friendliness	Assistance of the specific needs in physiologically based pharmacokinetic (PBPK) modeling.	Support of model building, hypothesis testing, user-defined output variables, data import from SBML, data export to Excel.
Application specificity	Software in terms of a PK-specific language.	Application-motivated design of underlying software structure; compounds, species, organs, dosing schemes, simulation objects as typical building blocks.
Modularity	Characterization of the overall PK model in terms of simple, independent modules that can be used in multiple contexts.	Extendable list of modules (physiological processes such as binding, transfer, metabolism, metabolic pathways, etc.) in a model basis. Definition of each full-body PK model in terms of the underlying physiological processes.
Orthogonal design and flexibility	Separation of model constituents that are independent of each other.	Independent definition of compound-specific data, species-specific physiological parameters and mathematical models to archive largest flexibility.
Openness	No limitation for adding new types of parameters, compounds, organs, species, physiological models, etc.	Easily extendable lists of parameters, compounds, organs, species, physiological models, dosing schemes, etc.
Transparency	No hidden calculations or parameters.	All parameters and models are directly assessable and editable.
Computational efficiency	Use of fast and accurate numerical techniques.	Integration of state-of-the-art numerical algorithms.

offset the effects of drugs and predict systemic side effects. Many of the necessary foundations have nearly been laid; however, there is no flexible application-specific and user-friendly software tool available that really supports this process in an open way.

The virtual laboratory approach

In mathematical terms, a PBPK model consists of a set of differential equations describing the ADME–Tox processes of one or more compounds in a system of more than a dozen organs, each

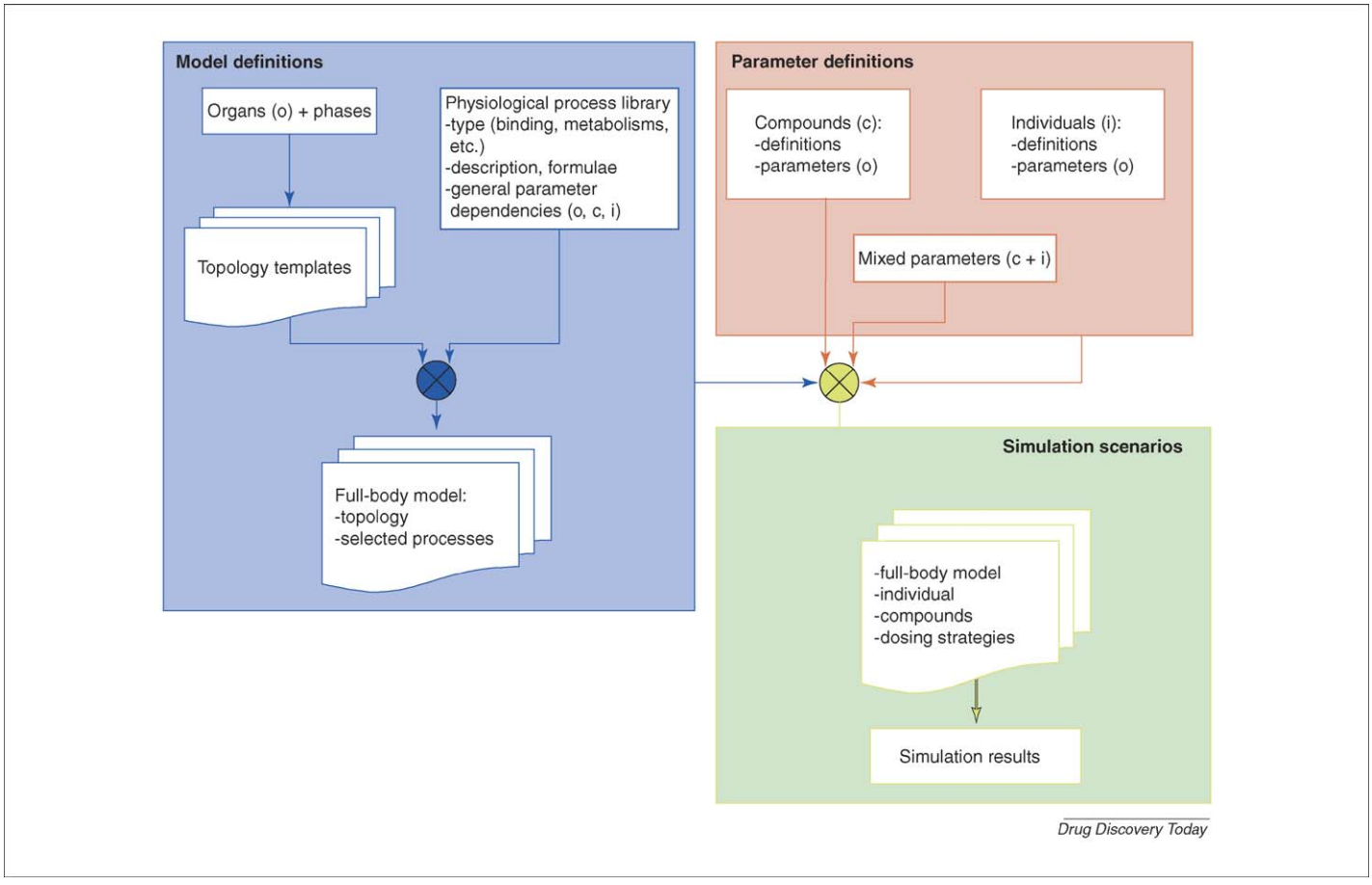

FIGURE 1

Modular structure of MEDICI-PK. All definitions, models and parameters can be entered and changed by the user and are not limited. Based on the defined framework (topology, model library, list of substances and individuals) components of a model form a so-called 'simulation object' describing one simulation scenario. A project might consist of an open list of all shown structures; simulation results from totally different models can easily be compared.

being possibly further subdivided into several phases (e.g. erythrocytes, plasma, interstitial space and cellular space). Coding such a system in a programming language is possible, however cumbersome and error prone, and a subsequent extension or change of the model will be even more complicated. For the design of a virtual laboratory it is obvious that, on the one hand, the software tools should allow for the input of models in a very open way yet, on the other hand, should not force the user to enter the whole set of differential equations. Thus, an implementation of a static model is prohibitive because it contradicts the requirement of openness for the software tool; furthermore, a purely equation-based approach contrasts with the requirement of user-friendliness. However, unfortunately these are two typical approaches often encountered in engineering software packages. Instead, the requirements on openness and user-friendliness have to be fulfilled by the use of a sophisticated modular structure implemented by means of modern software concepts [28].

The virtual laboratory should allow the implementation of a hierarchy of models suitable for the integration of as much knowledge (data) as is available into the model (i.e. few *in vitro* data at the beginning, followed by more-detailed *in vitro* data and *in vivo* data later on). At any point, the model should be completely transparent; there should be neither hidden calculations nor hidden parameters. From our experience, this is an important point because transparency is a prerequisite for confidence in the modeling process. In addition, as stated in Ref. [8], 'software producers need to improve transparency and state clearly the assumptions underlying their predictive approach'. Moreover, the integration of systems biology data (e.g. from the target-finding and validation process) should be possible, proceeding towards the simulation of biomarkers and effect-related processes. Of course, the use of state-of-the-art numerical algorithms and the possibility to analyze, easily and flexibly, the simulation output and the comparison of different models on the basis of their simulation results is self-evident. This is important for the a posteriori validation of early in comparison with refined models, as well as for the design of new models (e.g. for oral absorption). A modular structure should facilitate modeling of drug–drug interactions and support the development of disease-specific models for the design of virtual patients (humans as well as animals) that can be exploited in the drug discovery process.

MEDICI-PK

The design principles and concepts, derived previously in this review, have been realized for the first time in the virtual

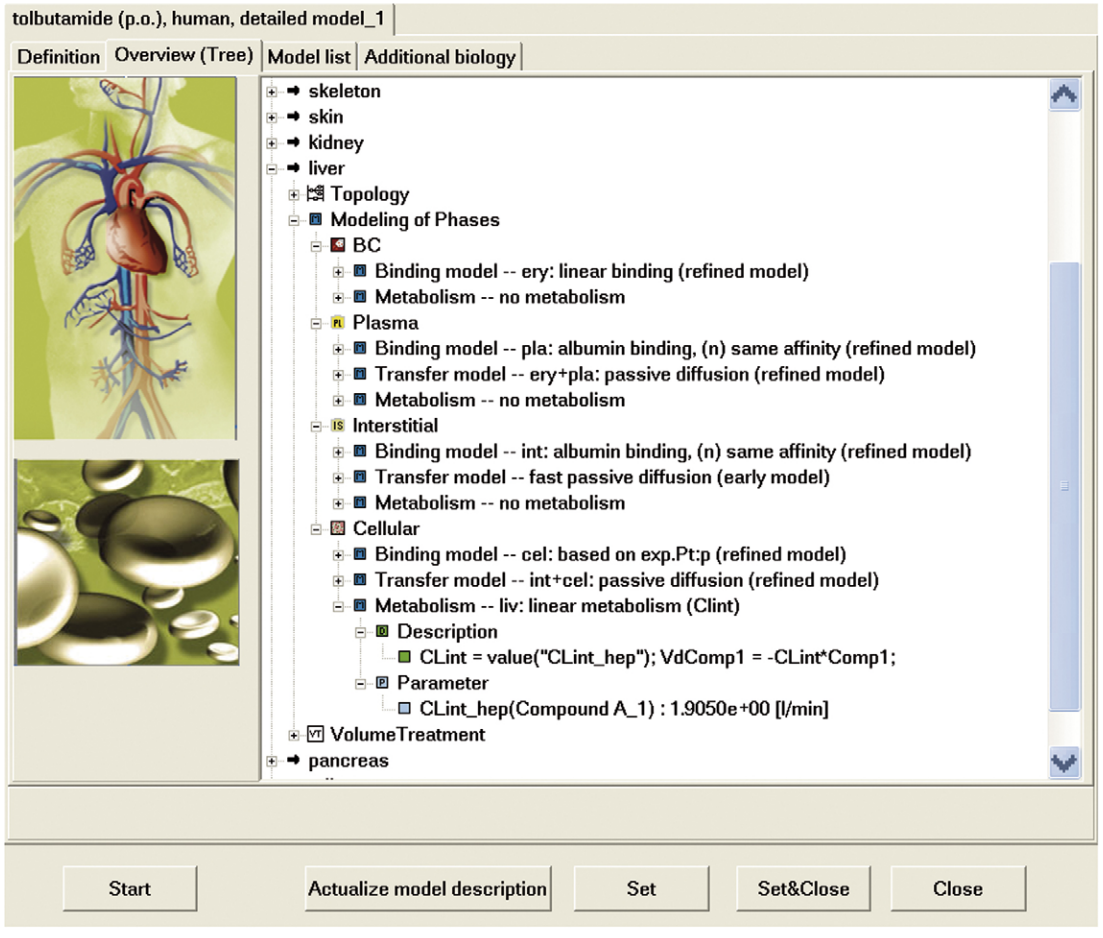

FIGURE 2

A Medici-PK simulation object summarizing all settings and parameters of a single simulation scenario. All details are assessable and can be changed in the model tree.

laboratory MEDICI-PK (see Table 1), demonstrating its feasibility and illustrating the advantages and benefits of the concept. The underlying structure can be seen in Figure 1. A crucial point is the so-called orthogonality of the model and the parameters. The compounds and the individuals (representing either a typical, healthy species or a disease type) are basically a collection of parameters and the source of input for the models. The models, however, are established independently from the compound and the individual. They are based on typical physiological processes, such as protein binding, diffusion across the membrane, metabolism, and so on. Each of these typical processes can be represented by a set of (differential) equations, without setting up the whole-body model. This guarantees that the underlying physiological processes are accessible, extendable and fully transparent. When performing a case study, one simply has to choose the underlying whole-body physiologically based model, the individual (and therefore the species) and the compound (Figure 2). This way, the greatest flexibility is retained. For instance, simulating the same compound with the same model for a different species requires just two 'clicks' (assuming, of course, that the necessary model parameters are available). To give another example, assume we want to simulate a prodrug, its metabolite (the drug) and a potential interaction of the drug with another compound in the kidneys (thinking of oseltamivir, oseltamivir phosphate (Tamiflu) and probenecid [29,30]). Then, in a first step one needs to choose a model, an individual and one of the compounds at a time to simulate their PK. Now, in a second step, the interaction needs to be introduced into the model. This is realized by defining two, new basic physiological models: (i) metabolism in the liver, taking explicitly the production of the metabolite into account; (ii) competitive excretion in the kidneys (to build this model one can use the insight from competitive metabolism). Choosing these two basic physiological models in the whole-body PBPK model, the overall interaction of the three compounds can be studied by simulation. This nicely illustrates how easily interactions of different compounds can be integrated into the model.

Several different models and simulation studies have already been realized in MEDICI-PK in this way (e.g. a model for Cyclosporine A [31], a detailed model on the PK of tolbutamide and a generic PBPK model for early drug development [5,32]). Immediately, extensions of the existing models have been studied (Figure 3), for example incorporation of metabolites, comparison of different binding and tissue distribution models, drug–drug interaction studies, and comparison between different species (human, rat, and so on).

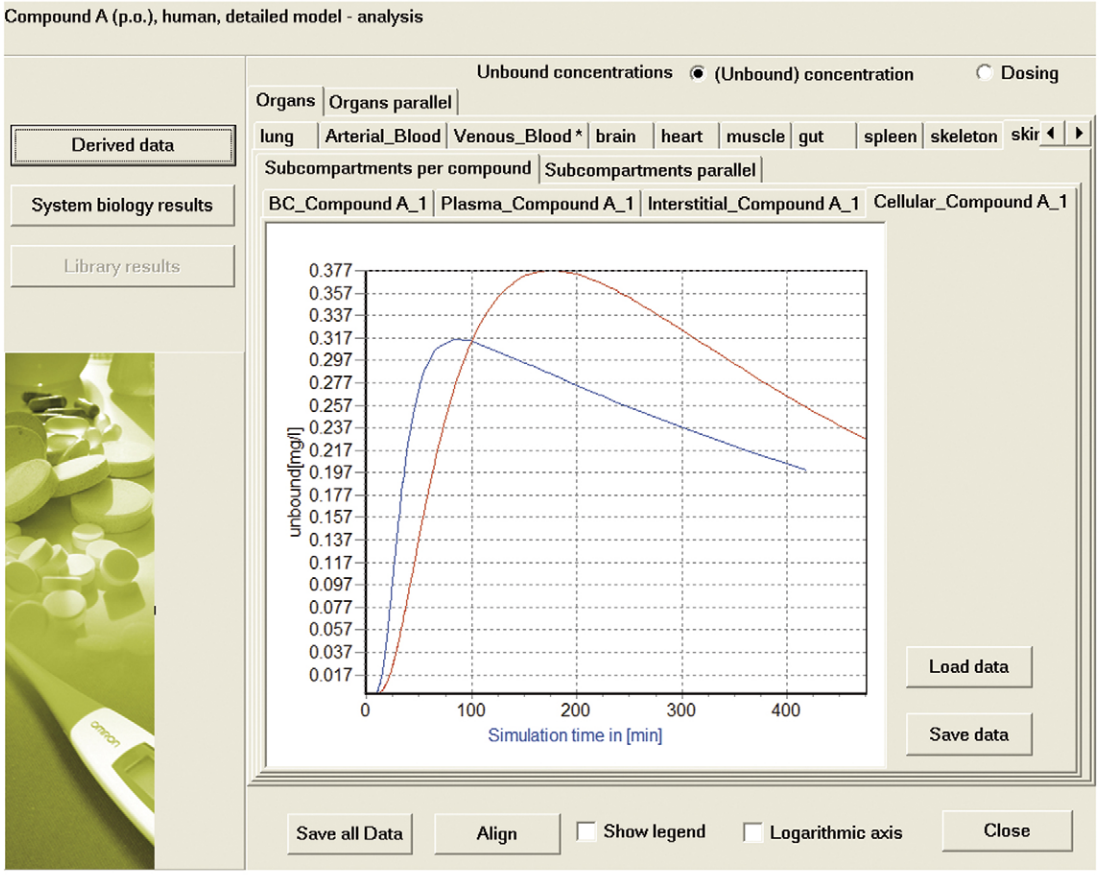

FIGURE 3

Typical simulation output of a MEDICI-PK case study. The results for all organs and phases can be visualized and compared. The graphic shows concentration time curves, where the blue line corresponds to the results obtained by using a more refined model in comparison with the result of a coarser model (red line).

Conclusion

This article presents the design principles and concepts of a virtual laboratory approach - implemented in the program package MEDICI-PK – that supports *in silico* modeling and simulation in PK and pharmacodynamics (PD). The concept is especially tailored to serve the needs in drug discovery. Based on our experience in other fields of chemistry and biology we see a high potential for *in silico* PK and PD approaches in the drug discovery and development process, if properly realized. When starting the modeling and simulation activities, there will soon be a first benefit: modeling eventually brings together people with different backgrounds and different company affiliations, thereby increasing the flux of information across different process stages (in this case ideally between research, development and the clinical phase). Moreover, in the process of developing an appropriate model, important modeling questions have to be addressed. What are the most relevant physiological processes and mechanisms? Is there any detailed knowledge about these processes or are they (partially) unknown? Are there competing hypotheses about important mechanisms? A further benefit is the integration of *in silico*, *in vitro* and *in vivo* data into a coherent framework by which they (model and data) undergo a kind of consistency check. Once a first model has been established, hypotheses can be generated and tested by simulation. Having gained some confidence in the model, predictions generated by simulation studies will influence the design of future experimental studies (*in vitro* and *in vivo*). As can be seen in many different fields, this feedback loop is often the beginning of a substantial change in the quality of models and understanding of the processes of interest.

It is important to note that building a comprehensive and predictive model usually cannot be realized in a few days. The decision to do modeling implies a long-term directive at best. It is a process gaining and accumulating new insights from the very beginning, but the final model will hardly look like the first attempts or approaches published elsewhere. For PK modeling this means that a compound should be continuously monitored *in silico* along its way through research, development and the clinical phases. Once established, a good model can be the starting point for new drug candidates, thereby decreasing the amount of work to be invested per compound and ensuring an information flux back from the (data-rich) clinical phase to the target-finding (or validation) and lead-generation phase.

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