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COMBINING SYSTEMS BIOLOGY WITH PHYSIOLOGICALLY BASED PHARMACOKINETICS TO SUPPORT THE UNDERSTANDING OF DRUG EFFECTS[1]

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Abstract: To increase the efficiency of drug discovery and development, modelling and simulation in pharmacokinetics and disease is gaining increasing attraction. Due to recent advances in physiologically based pharmacokinetics regarding the prediction of key parameters, it is now possible to predict concentration-time profiles in different spaces of various organs for large classes of compounds—even prior to the performance of any *in vivo* studies. The field has now reached a status that allows for ample exploitation on a much broader scale. Combined with systems biology models of cellular response at the target site this will result in more realistic mechanism based pharmacokinetic/dynamic models with a wide spectrum of important applications in current drug design, including e.g., identification and ranking of biomarkers candidates, *time resolved* analysis of drug-target interactions, support in prioritizing compounds, and the study of physiological variability and important sub-populations. First steps of this combined approach are illustrated for diabetes mellitus type 2 treatment with tolbutamide, and the implications on the design requirements for the systems biology models are discussed.

Keywords: physiologically based pharmacokinetics, drug discovery and development, drug monitoring, tolbutamide, insulin, diabetes mellitus type 2, pharmacodynamics, biomarker, complex systems, generating insight

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1. INTRODUCTION

A major reason for attrition in current drug development is the failure to demonstrate efficacy in late stage clinical development (Kola and Landis, 2004). Very often, the reason is the lack of predictive and/or timely (animal) models of clinical outcome. As a consequence, efforts are made to consider predictive tools of efficacy as early as possible, resulting in a boosting interest in biomarkers that in broad terms are defined as easy accessible molecular markers which correlate with the status or progression of the disease (Frank and Hargreaves, 2003). Biomarkers might also help to identify the relevant sources of pharmacokinetic or pharmacodynamics variability that contributes to the variable clinical outcome in certain groups of patients (Kloft *et al.*, 2006). To further understand and elucidate biomarkers, *in silico* are increasingly recognized as a valuable tool.

In silico approaches are increasingly recognized by pharmaceutical companies as a very valuable tool (Beresford *et al.*, 2002). An important class are physiologically based pharmacokinetic (PBPK) models. They allow to *integrate* data from different sources (Lüpfert and Reichel, 2006), and can provide links to systems biology models of cellular response. This type of approach offers the opportunity to generate *expectations/hypothesis* about future experiments based on the current underlying assumptions and data. Recently, crucial advances have been made in PBPK modelling. These regard the prediction of key input parameters (so-called tissue partition coefficients) based on readily measurable *in vitro* compound-specific data (Poulin and Theil, 2000; Rodgers and Rowland, 2006) as well as the modularisation of the PBPK models in terms of the underlying physiologically processes (von Kleist and Huisinga, 2007). As a result PBPK modelling is now much more accessible.

The integration of systems biology into the drug discovery process offers a new perspective (Ekins *et al.*, 2005; Butcher *et al.*, 2004; Hunter and Borg, 2003; Wolkenhauer *et al.*, 2007). In particular when coupled to physiologically based pharmacokinetic models, a wide range of important applications arise: It allows (a) to study processes that are often not accessible by experiments performed in clinical *in vivo* studies, (b) to make predictions solely based on the assumptions and data integrated into the model, (c) to identify and rank biomarker candidates, (d) to analyse drug-target interactions in a *time resolved* way, (e) to prioritise compounds based on pharmacokinetic as well as pharmacodynamic *in silico* characteristics, (f) to study the influence of physiological variability and the peculiarities of important subpopulations, such as patients with special characteristics like children or poor-metabolisers, (g) to guide in hypothesis generation, clinical study planing and optimization. Of course, *in silico* should be seen within the orchestra of *in vitro* and *in vivo* methodologies. Only the rationale combination of all available techniques will eventually guarantee progress in drug discovery and development, being always aware of the advantages and, equally important, of the limitations of the methods used.

In this paper we illustrate a combined systems biology-pharmacokinetics approach in application to diabetes mellitus type 2 treatment with tolbutamide. We introduce the necessary ingredients, present first results and discuss lines of necessary further research, which clearly demonstrate how both fields may fruitfully interact with each other.

2. DIABETES MELLITUS & TOLBUTAMIDE-INSULIN-GLUCOSE DYNAMICS

The literature dealing with mathematical modeling for diabetes type 2 is abundant, focussing on the different aspects of the disease, including glucose and insulin dynamics (e.g. (Keener and Sneyd, 1998; Fall *et al.*, 2002) and references therein). A simplified model for the release of insulin from the pancreatic β-cells was proposed by Maki and Keizer (Keener, 2001; Maki and Keizer, 1995) and will be discussed below. Sulfonylureas, such as tolbutamide, are oral antidiabetic drugs that increase insulin release from the pancreatic β-cells and thereby increase glucose uptake from the blood.

Our interest in diabetes mellitus modelling was motivated by a clinical study, in which the effect of polymorphisms in drug metabolizing enzymes on the glucose insulin metabolism was analyzed (Kirchheiner *et al.*, 2002). In the study, the plasma concentrations of tolbutamide, glucose and insulin were measured. While there was a good correlation between the activity of the metabolising enzymes and the drug pharmacokinetics (as expected), surprisingly no correlation between the drug concentration and the effect on insulin and glucose plasma levels could be found, which gave rise to different speculations.

Insulin is secreted from the pancreatic β-cells via exocytosis in an oscillatory fashion, stimulated by glucose metabolism within the β-cell. Glucose is taken up by a family of GLUT-type transporters; secreted insulin affects the transport of glucose into the cells by activating GLUT-1 transporters and inactivating GLUT-2 transporters. Thus, there is both positive and negative feedback. Maki and Keizer proposed a mathematical model to study the interactions in a cleverly

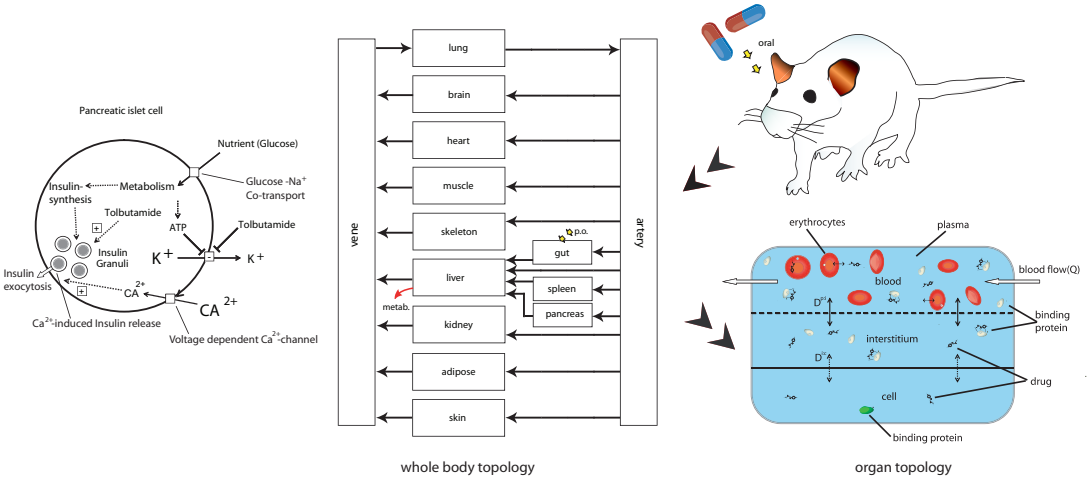

Fig. 1. The leftmost picture shows a β-cell model of insulin secretion including Tolbutamide-target interactions. An illustration of a physiologically based whole body pharmacokinetic model is shown in the middle and on the right.

designed *in vitro* experiment (Maki and Keizer, 1995; Keener, 2001). The setup involves extracellular glucose, extracellular insulin, and intracellular glucose. It further incorporates a flow through the bed of cells. The system of differential equations is given by

$$\frac{\mathrm{d}}{\mathrm{d}t}G^i = -R_1 - R_2 - k_0(G^i - G_0) \qquad (1)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}I^i = R_s - k_0(I^i - I_0) \qquad (2)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}G^c = R_1 + R_2 - R_m. \qquad (3)$$

The concentrations G^i,I^i and G^c correspond to the interstitial glucose and insulin concentrations, as well as the intracellular glucose concentration. The terms R_1 and R_2 represent the uptake rates of glucose through GLUT-1 and GLUT-2 transporters, R_s and R_m denote the rate of insulin secretion and the rate of glucose metabolism. The parameter k_0 represents the inflow with G_0 and I_0 representing the inflow concentrations (which could be interpreted as the capillary concentrations). The system is coupled through the definition of the rates R_i. All parameters and a discussion of the model can be found in the original articles (Maki and Keizer, 1995; Keener, 2001).

Although simplified, this model may serve as a good starting point for the integration of the insulin-glucose interaction into a physiologically based pharmacokinetic model. The model has been developed after *in vitro* experiments with intact β cells have been carried out. It is worth mentioning that in view of integrating systems biology models into PBPK models, the former should allow for a physiological or mechanistic interpretation (see also Sec. 6).

Table 1. Tolbutamide specific pharmacokinetic parameters.

	rat [a]	human		
tissue	$K^{t:up}$	$K^{t:up}$	param.	value
lun	0.76	1.74	fu^p	0.093 [g]
brn	0.3	3.11	CL (1*/1*)	16 [b e]
hea	0.83	1.35	CL (1*/3*)	10 [b e]
mus	0.4	0.87	CL (3*/3*)	2.7 [b e]
gut	0.36	1.03	pK_a	5.43
spl	0.59	1.39	MAT	34.2 [d e]
ske	0.48 [f]	1.28	CV^2_A	0.34 [e]
skin	0.68	3.05	$\log P_{o:w}$	2.4 [i]
kid	0.67	1.53	f_{bio}	0.9 [e]
liv	0.93	1.64		
pan	0.89	1.46		
fat	0.39	0.92		
ery	1.35	1.35		

3. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

A physiologically based pharmacokinetic (PBPK) whole body model is a special type of compartmental model, in which the compartments represent anatomical volumes, such as organs or tissues, interconnected by the blood flow (e.g., Theil *et al.*, 2003, Nestorov, 2003). The conceptional representation of a 14 compartment PBPK model is shown in Fig. 1 (middle and right). In mathematical terms, PBPK models are coupled systems

[a] reference: (Sugita *et al.*, 1982)
[b] plasma clearance with unit [mL/min], based on a 80 kg human
[c] unit [μM]
[d] unit [min]
[e] reference: (Kirchheiner *et al.*, 2002)
[f] determined *in silico* by the method developed by Rodgers et al. (Rodgers and Rowland, 2006) using *in vitro* parameters
[g] reference: (Sawada *et al.*, 1985)
[h] reference: (Kamp *et al.*, 2003)
[i] reference: http://www.syrres.com/esc/kowdemo.htm

of ordinary differential equations, parameterized by physiological and compound-specific parameters. Due to recent advances in the determination of key parameters (Poulin and Theil, 2000; Rodgers *et al.*, 2005; Rodgers and Rowland, 2006), parametrization of generic PBPK models is now possible for many classes of compounds, even prior to the performance of any *in vivo* experiments.

Recently, we have introduced a modular approach to physiologically based pharmacokinetics (von Kleist and Huisinga, 2007), in which each compartment is further sub-divided into the four phases: erythrocytes, plasma, interstitium and cellular space (see Fig. 1). Many physiological processes in pharmacokinetics are now accessible for a mechanistic description at this resolution. In addition, we have introduced advanced software concepts that enable a modular approach to pharmacokinetic/pharmacodynamic modeling, allowing for the user-friendly integration of systems biology models (Huisinga *et al.*, 2006; Telgmann *et al.*, 2006). These have been realized in the virtual lab MEDICI-PK (CiT Rastede/Germany) that has been used for the simulation studies herein.

4. TOLBUTAMIDE DRUG CHARACTERISTICS

Tolbutamide is a weak mono-protonic acid that rapidly passes cellular membranes by free diffusion (Kamp *et al.*, 2003). It is extensively bound to albumin in the plasma and interstitial space (Jakoby *et al.*, 1995). Tolbutamide is approximately linearly cleared from the body by metabolism, mainly by the liver enzyme CYP450 2C9. Compound specific parameters to model tolbutamide pharmacokinetics are displayed in Table 1—the relevant physiological parameters can be found in (Poulin and Theil, 2000; Rodgers and Rowland, 2006). Tolbutamide stimulates insulin secretion from pancreatic β-cells. Its main target is the sulfonylurea receptor of the β-cells, a component of the ATP-sensitive potassium channel (K_{ATP}). This channel plays a major role in controlling the β-cell membrane potential. At rest, the K_{ATP} channel is open and maintains the membrane potential at a hyperpolarized level that prevents insulin secretion (McClenaghan and Flatt, 1999). Closure of the K_{ATP} channel by glycose metabolism or sulfonylureas (Ashfield *et al.*, 1999) causes membrane depolarization which, in turn, triggers Ca^{2+} influx through voltage gated Ca^{2+} channels and thereby stimulates the exocytosis of insulin-containing secretory granules (Geng *et al.*, 2003), see Fig. 1(left picture).

A concentration of 8-9 [mM] glucose causes an insulin release (McClenaghan and Flatt, 1999), which is equivalent to a 90 % closure of the

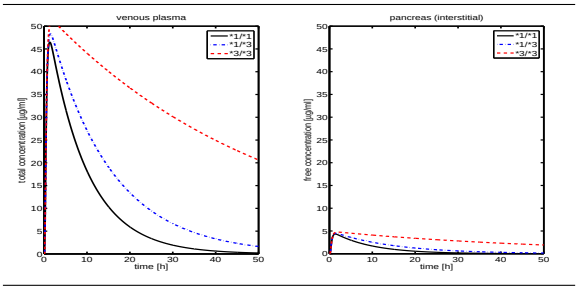

Fig. 2. MEDICI-PK Simulation results showing the influence of polymorphism on tolbutamide concentrations: Fast metabolizer (1*/1* genotype, solid line), intermediate metabolizer (genotype 1*/3*, dashed-dotted lines), and slow metabolizer (genotype 3*/3*, dashed lines). The left panel shows the concentration-time profiles in the venous plasma (experimentally accessible), while the right panel shows the free (unbound) concentration in the interstitial compartment of the pancreas, which is the relevant concentration in the effect compartment.

K_{ATP} channels. A concentration of 15-25 [μM] tolbutamide has the same effect (Jonkers *et al.*, 2001).

5. *IN SILICO* STUDY OF THE EFFECT OF POLYMORPHISMS

As described in Sec. 2, we are interested in the implications of polymorphism in the metabolising enzyme 2C9 on tolbutamide pharmacokinetics and its effects on insulin and glucose levels. This is the first step towards analysing the clinical study outcome performed by (Kirchheiner *et al.*, 2002). We set up a physiologically based pharmacokinetic model using the parameters listed in Table 1. Convincing agreement with experimental results could be established (e.g., comparison with experimental results by Sugita *et al.*, 1982 for rats, data not shown). The simulations for humans shown in Fig. 2 clearly illustrate the impact of polymorphism on tolbutamide pharmacokinetics. In particular, the genotype related to poor–metabolisers (*3/*3) shows prolonged exposure to tolbutamide.

The above study only considers the pharmacokinetics of tolbutamide. Concerning the insulin-glucose interactions, the predictions according to the Maki and Keizer model are shown in Fig. 3. The uptake of glucose from the inflow and its subsequent metabolism triggers the release of insulin. Positive and negative feedback mechanism result in oscillatory behaviour.

The next step is the integration of a systems biology model of tolbutamide-glucose-insulin interaction in the interstitial and cellular space of

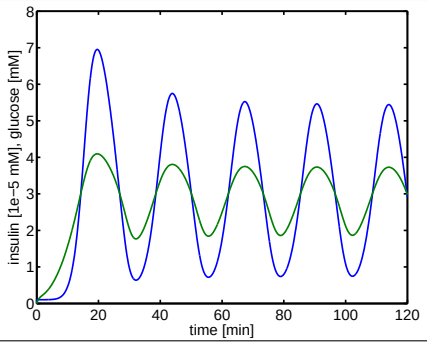

Fig. 3. Oscillatory release of insulin (solid, blue line) triggered by intracellular glucose (dashed, green line) as predicted by the Maki and Keizer model based on a constant glucose inflow of 12 [mM].

the pancreas, as shown in Fig. 1 (left picture). This would allow to study the implications of the different drug levels on the insulin and glucose levels. Maki and Keizer have shown that oscillatory behaviour and insulin release is sensitive to the incoming glucose concentration. Similar behaviour can be expected in relation to tolbutamide concentrations. Consequently, only the coupling of the full time-dependent pharmacokinetics to a cellular model of insulin release would correctly account for the complex interaction. Due to the modularity and resolution of the chosen PBPK model, such a combined model is now accomplishable.

6. DISCUSSION & OUTLOOK

We have established a physiologically based pharmacokinetic (PBPK) model for tolbutamide that serves as a starting point for the subsequent integration of pharmacodynamic processes, like the model by Maki and Keizer (see Sec. 2). There is much work to be done to model, simulate and finally interpret the implications of polymorphism on the effect on glucose and insulin levels. Our studies should rather been seen as an illustrative example for the rich field of applications of systems biology in future drug discovery and development, when tightly coupled to mechanistic pharmacokinetic models.

This combined approach has also several direct implications on the way systems biology models of cellular response should be constructed. Ideally, the models should be parameterized, e.g., by correspondingly designed *in vitro* experiments. Moreover, the cellular model and the corresponding parameters should allow for a physiological interpretation. This is typically the case for PBPK models where, e.g., tissue partitioning is predicted based on compound-specific parameters combined with tissue decomposition data, or liver metabolism is estimated via *in vitro* assays using hepatocytes. The overall model then predicts the complex interaction of all its "simple" constituents—rather than being fitted to experimental data to reproduce those data. Allowing for physiologically interpretation is a main advantage of PBPK models over empirical pharmacokinetic models. This offers, e.g., the possibility to extrapolate between different species, which empirical models rarely do or is rather difficult to perform with.

When analyzing detailed cellular models, e.g., involving a drug target molecule and downstream effects, constant drug concentrations are often assumed as an input signal. Here PBPK models offer the possibility to integrate more realistic drug concentration profiles as stimulating input signals, thus accounting for the "neglected" environment.

The tolbutamide-insulin-glucose study has much further research to offer: The question of possible intracellular targets of tolbutamide, besides the well known effects of tolbutamide on the K_{ATP} channel of the pancreatic β-cells could be analyzed *in silico*. The question of how glucagon influences the glucose insulin metabolism needs to be addressed, too. Glucagon is secreted by the pancreatic α cells and it is known to have a counteracting effect compared to insulin. Experimental results suggest a direct impact of tolbutamide on glucagon secretion that is both inhibitory and stimulative and comparable to that of glucose. In order to explain the experimental findings of the mentioned clinical study (Kirchheiner *et al.*, 2002), it might also be necessary to take the electrophysiology of the pancreatic α cells into account (Diderichsen and Gopel, 2006).

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