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Using Petri nets for experimental design in a multi-organ elimination pathway

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ABSTRACT

Genistein is a soy metabolite with estrogenic activity that may result in (un)favorable effects on human health. Elucidation of the mechanisms through which food additives such as genistein exert their beneficiary effects is a major challenge for the food industry. A better understanding of the genistein elimination pathway could shed light on such mechanisms. We developed a Petri net model that represents this multi-organ elimination pathway and which assists in the design of future experiments. Using this model we show that metabolic profiles solely measured in venous blood are not sufficient to uniquely parameterize the model. Based on simulations we suggest two solutions that provide better results: parameterize the model using gut epithelium profiles or add additional biological constrains in the model.

1. Introduction

Genistein is a soy metabolite with estrogenic activity that may result in (un)favorable effects on human health (for a review see [1]). Elucidation of the mechanisms through which food additives such as genistein exert their beneficiary effects is a major challenge for the food industry. A better understanding of the genistein elimination pathway could shed light on such mechanisms. Parts of this pathway are hosted by specific organs (including the small intestine, gut, liver, and kidney). Metabolite degradation products travel between these organs and eventually are secreted through the gut or kidney. Although many nutrikinetics studies have been conducted to explore the genistein multi-compartment elimination pathway in human and animal models, relatively few of its details are known and, consequently, the precise metabolic pathways and routes remain to be established. In this work we therefore do not consider detailed metabolic reactions involved in the elimination pathway (which are largely unknown) but focus on the routes of degradation products through the network of compartments (the involved organs and blood).

Mathematical modelling helps to gain a more detailed understanding of the genistein elimination pathway but this requires a model describing this system in sufficient detail. However, Petri nets are able to use incomplete and/or imprecise information to reconstruct system's behaviour. Petri nets developed by Carl Adam Petri provide a generic approach for modelling of concurrent systems [2]. A Petri net is a bipartite graph with two types of nodes – places and transitions. In biological applications, places generally represent biological entities such as molecules, genes and enzymes. Places contain tokens that reflect, for example, metabolite concentrations or gene expression levels. Transitions represent relations between biological entities such as enzymatic reactions or metabolite transport. A Petri net simulation results in a time-dependent redistribution of tokens reflecting system dynamics. Simulation of the Petri net model implies that we select and 'fire' a specific transition resulting in tokens being moved from one place to the next. The firing rules define which transition fires and the number of tokens subsequently transferred. This, together with the topology of a Petri net model, results in a qualitative representation of the system's dynamics.

Petri nets have become a popular tool for studying biological networks such as metabolic networks [3]. The review paper of Baldan et al. explains how metabolic pathways have been represented and modelled with Petri nets [4]. The authors also discuss various ways to use Petri nets for modelling network topology structures such as negative feed-back loops and inhibition. Modelling network topology with Petri nets has been shown to give qualitative biologically relevant insights about the dynamics of biological systems [5,6]. A quantitative analysis of biological network dynamics required further extension of

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Petri nets in a way similar to mathematical modelling using ordinary differential equations (e.g., [7]). Such Petri nets require knowledge of kinetic parameters. However, it has been shown that network dynamics might be determined by using only network topology [8,9]. For example, Ruths and co-workers [10] assumed that network connectivity is the most significant determinant of the signal propagation and that discarding kinetic parameters from the model still results in model outcome that agrees with experimental data in the majority of cases. A similar example involved the use of Fuzzy Logic to reconstruct the topology of a cell signalling network from gene expression data in silico [11].

Petri net models can also assist in designing new wet-lab experiments to further characterize the system under investigation. In this paper we demonstrate how a Petri net model representing the human genistein multi-organ elimination network fits this purpose (Fig. 1). This model describes various routes involved in the elimination of genistein after dietary exposure to this compound. Each transition of metabolites within or between organs is associated with a fraction (F) that indirectly represents its relative flux. The network topology and fractions define a model configuration and allow the simulation of time-resolved metabolite relative concentration profiles for different organs given an amount of genistein input G(I) administered to an individual (Fig. 2A). The challenge, however, is to estimate fractions from measured profiles. In this work we use concentration profiles from LC-MS venous blood measurements obtained in a nutrikinetics study in which healthy volunteers were exposed to dietary genistein [12]. The estimation of fractions is challenging because given current domain knowledge and available data, the genistein elimination pathway is insufficiently constrained and, therefore, ambiguous in terms of model configurations (i.e., sets of fractions) being in agreement with experimental data. Therefore, we used our model as an experimental design tool to investigate which additional information (data or prior knowledge) would be required to further constrain the system to allow accurate estimations of the fractions. In particular, we investigated if additional metabolite profiles and additional constrains (e.g., fixing fractions associated with excretion transitions) would result in better parameter estimates.

To answer these experimental design questions, we used simulated annealing (SA) to estimate fractions from experimental or simulated metabolite profiles.

2. Results

2.1. Fraction estimation from simulated reference profiles for all places

We first explored if fractions can be correctly estimated based on concentration reference profiles simulated for all places in our model (Fig. 1). Consequently, we configured the Petri net with all fractions arbitrarily set to 0.5 except for fractions F2, F30 and F31 which were set to 0.34, 0.33 and 0.33 respectively (Fig. 2(A)). This fulfills the requirement that the fractions corresponding to outgoing transitions of a single place sum to one, and no preference for a specific transition is assumed (see Materials and Methods section). Then, we executed ten simulations with 1000 input tokens for place G(I). A simulation is terminated when all tokens left the Petri net. During simulations we recorded the number of tokens in each place to obtain the concentration reference profiles. Subsequently, using these simulated reference profiles we reconstruct the fractions through simulated annealing (Fig. 2(B)). The results from these reconstructions show that some fractions are precisely estimated (e.g., F1 and F7 were estimated within 2% of their true value), while other fractions showed large variability (e.g., F17, F16 deviated 90% of their true value; Fig. A.3). Note that all boxplots presented in this paper are sorted according to their estimation variances.

We defined three classes of relative estimation errors, which only can be calculated for estimates based on simulated reference profiles since the true fractions underlying the experimental data are unknown. A transition was classified as 'determinant' if the relative estimation error was less than 10%. The 'moderate' and 'flexible' classes correspond to estimation errors of 10–25%, and $>25\%$ respectively. Although simulated annealing may converge to sub-optimal solutions (fractions), we decided not to remove one or more runs with high(er) estimation errors (i.e. outliers in the boxplots) since this may lead to a biased result and, moreover, since this is also not possible for results based on experimental data due to unknown estimation errors.

To facilitate comparison with experimental data we defined three variance classes (low, medium, high, Fig. A.1). These classes are based on visual inspection of estimation variances observed from experimental data (Fig. A.2). Fig. 3 shows a visual representation of the estimation errors and variances in the context of our

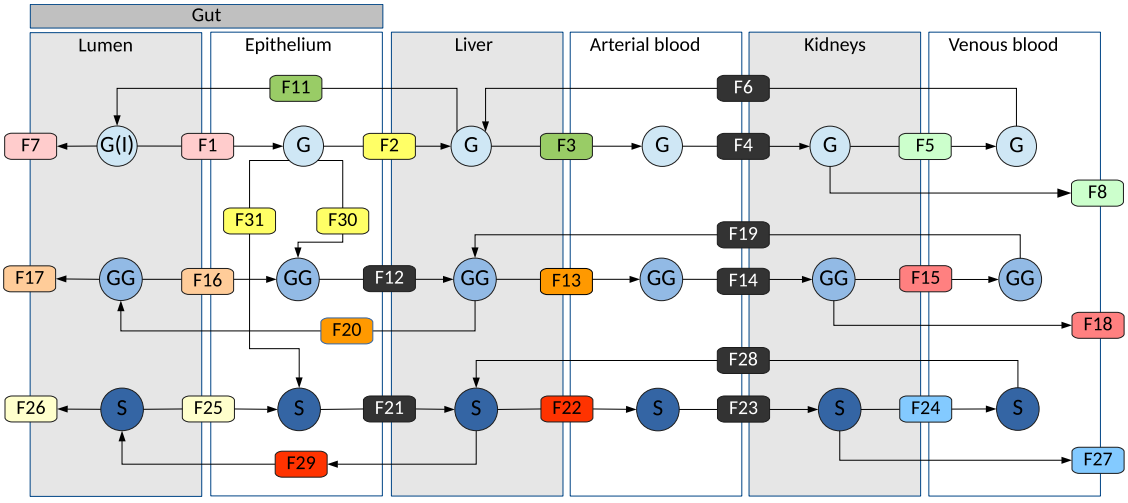

Fig. 1. *Petri net model of the human genistein multi-compartment elimination pathway.* This model includes three metabolites (G – genistein, GG – genistein-7-glucuronide; S – genistein-7-glucuronide-4-sulphate) that travel within and between six compartments (organs, blood). G(I) is the input place, which is set to 1000 tokens at the start of a simulation and represents the amount of genistein administered to an individual. Each transition is associated with a fraction (F) indirectly representing a relative flux. Associated fractions corresponding to outgoing transitions originating from the same place are shown in the same colour. Outgoing transitions associated with places without other outgoing transitions are shown in black. Six transitions (F7, F17, F26, F8, F18 and F27) represent the excretion of metabolites from the gut or kidney. (For interpretation of the references to colour in this figure caption, the reader is referred to the web version of this paper.)