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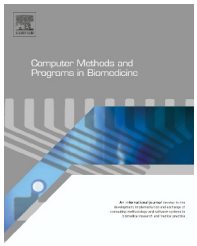

Structural identifiability analyses of candidate models for *in vitro* Pitavastatin hepatic uptake☆,☆☆

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ABSTRACT

In this paper a review of the application of four different techniques (a version of the similarity transformation approach for autonomous uncontrolled systems, a non-differential input/output observable normal form approach, the characteristic set differential algebra and a recent algebraic input/output relationship approach) to determine the structural identifiability of certain *in vitro* nonlinear pharmacokinetic models is provided. The Organic Anion Transporting Polypeptide (OATP) substrate, Pitavastatin, is used as a probe on freshly isolated animal and human hepatocytes. Candidate pharmacokinetic non-linear compartmental models have been derived to characterise the uptake process of Pitavastatin. As a prerequisite to parameter estimation, structural identifiability analyses are performed to establish that all unknown parameters can be identified from the experimental observations available.

1. Introduction

Pitavastatin is a drug used to treat hypercholesterolaemia. It shows active uptake into hepatocytes, mediated mainly by Organic Anion Transporting Polypeptide (OATP) 1B1 [1,2]. It is desired to investigate the nonlinear kinetics of in vitro hepatic uptake of the OATP substrate, Pitavastatin, and quantify the mechanisms present both structurally and numerically. Experiments utilising the 'oil spin' methodology described by [3] have been designed at AstraZeneca to investigate the nonlinear kinetics of *in vitro* hepatic uptake. Six candidate models are proposed to characterise the uptake process. In order to perform parameter estimation and compare the models to ascertain those most suitable for predictive purposes, it is a necessary requirement to first ask "do the observations uniquely determine the unknown model parameters?". In the models derived, as in most biomedical systems modelling, the model parameters have biological meaning and it is desired to establish whether it is at all possible to estimate their values from experimental data. Techniques for structural identifiability analysis look to determine whether unknown model parameters have unique values given the available observation(s) [4].

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2. Structural identifiability

Structural identifiability analysis considers the uniqueness of the unknown model parameters from the input/output structure corresponding to proposed experiments to collect data for parameter estimation (under an assumption of the availability of perfect, noise-free data) [5,6]. This is an important, but often overlooked, theoretical prerequisite to experiment design, system identification and parameter estimation, since numerical estimates for unidentifiable parameters are effectively meaningless. If parameter estimates are to be used to inform about intervention or inhibition strategies, or other critical decisions, then it is essential that the parameters be uniquely identifiable. Such analysis is highly relevant to large-scale, highly complex systems, which are typical in chemical kinetics and systems biology [7,8]. It is important to note that an *a priori* structurally identifiable model does not necessarily guarantee a posteriori numerical parameter identifiability, for example [9], however it does greatly increase the confidence in the parameter estimation process for the given system observation.

Numerous techniques for performing a structural identifiability analysis on linear parametric models exist and this is a well-understood topic [5,10]. In comparison, there are relatively few techniques available for nonlinear systems (the Taylor series approach [11], similarity transformation based approaches [12,13], and differential algebra techniques) [14,15] and significant computational problems can arise for these, even for relatively simple models [16,17].

In this paper, four methods are reviewed: a version of the similarity transformation approach for autonomous uncontrolled systems [18], a non-differential input/output observable normal form approach [19], the characteristic set differential algebra approach [14,15], and a recently introduced algebraic input/output relationship approach [19]. Each approach is performed on all of the Pitavastatin pharmacokinetic models developed in order to ascertain whether the unknown system parameters can be identified uniquely or otherwise for the observation available and to compare their performance.

For a given output, an *unidentifiable* parameter can take an (uncountably) infinite set of values, whereas a *nonuniquely (locally) identifiable* parameter can take any of a distinct (countable) set of values. A parameter is *globally identifiable* if for a given output, it can only take one value.

If all of the unknown parameters are globally identifiable, the system model is structurally globally identifiable (SGI). In the case that all parameters are locally identifiable and at least one is non-uniquely identifiable then the model is structurally locally identifiable (SLI). In the case where at least one parameter is unidentifiable then the model is structurally unidentifiable (SU).

Due to the complex nature of the analytical approaches, a symbolic computational package, namely Maple 2010 (Maplesoft) [20], was used to perform the analyses.

3. Models

As described previously, Pitavastatin is a substrate of OATP, which actively mediates the transport of the drug across the hepatocyte membrane. Diffusion also takes place at the cell membrane, where the drug flows in and out of the cell according to the concentration gradient. These two mechanisms can be represented by the compartmental model shown in Fig. 1. In the model, each compartment represents a different component of the hepatocyte cell.

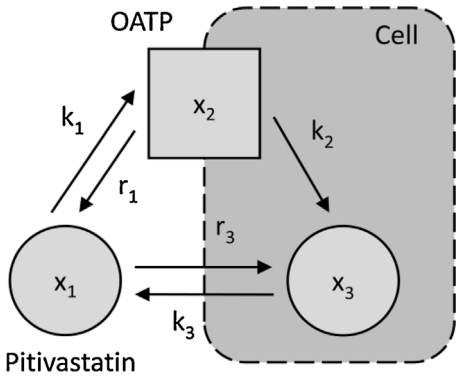

Fig. 1 – Basic conceptual model representation.

A known concentration of Pitavastatin can be added to the medium in which the hepatocytes sit at the beginning of the experiment (x_1). The substrate actively binds to OATP (x_2) and is mediated into the cell (x_3). Extracellular Pitavastatin (x_1) also flows into the cell (x_3) by diffusion with rate constants k_3 and r_3.

3.1. System equations

The system of ordinary differential equations describing the models is derived using classical mass-balance principles as per [4] for example. The corresponding model equations are given by:

$$\dot{x}_1 = k_3x_3 - r_3x_1 - k_1x_1(T_0 - x_2) + r_1x_2 \quad (1)$$

$$\dot{x}_2 = k_1x_1(T_0 - x_2) - (r_1 + k_2)x_2 \quad (2)$$

$$\dot{x}_3 = r_3x_1 - k_3x_3 + k_2x_2 \quad (3)$$

where T_0 is the total number of transporter binding sites on OATP. Here the unknown parameter set, p, is given by:

$$p = \{k_1, r_1, k_2, k_3, r_3, T_0\}. \quad (4)$$

The initial conditions are given by:

$$x_1(0) = D, \quad x_2(0) = 0, \quad x_3(0) = 0, \quad (5)$$

where D is the initial dose in μmol (1 million cells). The initial concentration of the medium in which the hepatocytes sit at the beginning of the experiment is known and given in μmol/L. The initial volume is 1 mL and 1 million cells are used thus the initial concentration is multiplied by a factor of 10^{-3} to convert it from μmol/L to μmol (1 million cells). x_1, x_2, x_3 therefore denote quantities in μmol (1 million cells).

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