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Review

Innovative tools in the individualized medical therapy for children with heart muscle disease

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ABSTRACT

Individualized medical therapy for children with heart muscle disease remains a challenge. Two innovative tools, physiologically based pharmacokinetic modeling and stem cell technology, have the potential to address these challenges. In this review, these technologies are introduced to provide the reader with a general conceptual overview and how they can help in guiding individualized treatment.

In recent years, there has been an explosion of knowledge about how the genetic makeup of patients governs their response to medical treatment. For heart failure patients or patients after heart transplantation, drugs such as ACE-inhibitors, AT1-receptors, β-adrenergic receptor blockers, aldosterone antagonists as well as immunosuppressives might be candidates for pharmacogenetic-tailored drug therapy. To maximize the advantage for the individual patient, novel treatment approaches integrate pharmacogenetic information into physiologically-based pharmacokinetic models to predict drug absorption, distribution, metabolism and elimination (ADME) in the individual patient. How these models are built and how they can help in guiding individual treatment protocols will be discussed and several examples presented.

Although population-based gene variant studies provide an important first step towards applying a pharmacogenomic approach to heart failure medications in children, the true connection between any gene variant and its effect on drug efficacy and toxicity in the clinical setting requires additional proof. Given the difficulty in transitioning past genome-wide association study (GWAS) data into clinical practice, it is important that each candidate gene variant be validated using a suitable model system. In the past, these model systems were limited to whole animal studies, where data interpretation was complicated by species differences. The lack of a human cardiomyocyte cell line had similarly prevented the translation of many *in vitro* studies to clinical practice. Recent advances in stem cell technology, including the generation of human cardiomyocyte cell lines from induced pluripotent stem cells (hiPSCs), provide a new platform to test drugs for both toxicity and therapeutic benefit.

1. Introduction

In recent years, there has been a rapid increase in our cumulative knowledge regarding the role of genetic determinants in explaining and predicting an individual patient's response to different medical treatments. Gene mutations and polymorphic variations of drug targets and/or metabolizing enzymes can even be the major determinant of the pharmacological response in certain patient populations [1]. If information about the genetic makeup of individual patients is taken into consideration, pharmacogenetically-tailored drug therapy would be a major step towards better therapy individualization for safer and more effective clinical outcomes [2].

The impact of a pharmacogenetically guided therapy is perhaps best illustrated in the field of oncology, where it has been shown to influence the clinical outcomes for many chemotherapeutic agents and, therefore, many are included in drug labeling information. For heart failure patients or patients after heart transplantation, drugs such as ACE-inhibitors, AT1-receptors, β-adrenergic receptor blockers, aldosterone antagonists as well as immunosuppressives might be candidates for pharmacogenetically tailored drug therapy, as many demonstrate extensive polymorphic variations in their pharmacodynamic targets and/or metabolizing enzymes [3]. The vast majority of the available clinical evidence supporting pharmacogenetically guided therapy in heart disease is obtained from retrospective studies; however, robust evidence from prospective randomized controlled trials supporting such an approach is available for some agents, such as the immunosuppressive drug tacrolimus [4].

In search for new approaches to achieve and/or support individualization of drug therapy, the so-called physiologically based pharmacokinetic (PBPK) models present themselves as innovative tools that can be

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incorporated into patient specific information and aid in improving current or future treatment strategies [5].

Another innovative tool has been made possible by recent advances in stem cell biology [6,7]. Researchers are now able to generate pluripotent stem cells (hiPSCs) directly from adult somatic (skin, fat, blood) cells obtained directly from patients. These reprogrammed cells can then be induced to differentiate into beating cardiomyocytes (hiPSC-CMs). This novel technology offers cardiovascular pharmacologists a unique opportunity to study the mechanisms of drug action directly in human cell lines. iPSC-CMs can be used in high-throughput platforms for screening drugs for efficacy and toxicity using cells from patients with various forms of cardiac diseases as well as from healthy controls.

In this application, HiPSC-CMs provide several advantages over previous model systems. One of the major limitations in cardiac pharmacology has been that human cardiomyocytes cannot be easily maintained in cell culture. Prior studies of human myocardium have usually been performed on samples obtained at the time of heart transplantation or left ventricular assist device implantation. Thus, these samples represent the end-stages of disease, making it impossible to determine which changes are primary mechanisms induced by a specific gene mutation or variant versus those that are secondary and tertiary effects induced by chronic neurohormonal stimulation.

Because of these limitations, most *in vitro* drug efficacy/toxicity studies have been performed on either transformed human cell lines or on rodent cardiomyocytes, which differ from mature human cardiomyocytes in many important characteristics, including sarcomeric structure, expression of contractile proteins, contraction rate and electrophysiologic function, making it difficult to extrapolate data from rodents to humans. This has led to many *in vitro* and *in vivo* findings that do not hold up in clinical translation to humans [8,9]. Studies of genetic cardiomyopathies are a particular challenge, given that the common human mutations in β-myosin heavy chain (MHC) cannot be readily duplicated in rodent models due to their dominant expression of α-MHC in the cardiac sarcomere. A mutation introduced into the α-MHC gene causes a different biomechanical alteration than when introduced into the β-MHC gene [10]. Thus, hiPSC-CMs arguably represent the best currently available *in vitro* model of cell function of the human heart [11].

As can be seen, PBPK models and hiPSC-CMs models are innovative techniques that offer different approaches of drug therapy individualization. We will review these technologies to provide the reader with a brief conceptual overview and how they can help in guiding individualized patient treatment.

2. Discussion

2.1. The Concept of Physiologically Based Pharmacokinetic (PBPK) Models

PBPK models construct "virtual subjects" in an *in silico* environment to investigate the fate of drugs in various body tissues and their changes due to the major pharmacokinetic processes: absorption, distribution, metabolism, and elimination. Here, our expansive knowledge of human anatomy and physiology is employed to represent the various organs and tissues of the human body as compartments connected together structurally *via* the circulation and mathematically by differential mass-balance equations [5]. These equations quantitatively describe drug movement between system compartments and incorporate physiological and drug-specific parameters. The former group characterizes the anatomical structure and physiological processes of the species being modeled, such as cardiac output, organ/tissue volumes and blood flows, tissue composition, surface area, pH values, and transit times for the gastrointestinal tract, whereas the latter group includes drug-specific information such as its physiochemical properties (molecular weight, lipophilicity, ionization, solubility, and plasma protein binding), permeability, and clearance pathways. Fig. 1 shows a simplified representation of the structure of PBPK models.

Because of this structuring concept, PBPK models can be used to perform "what-if" scenarios *a priori* and provide a mechanistic explanation of the impact of multiple intrinsic and extrinsic variables such as age, sex, renal and liver function, dosing regimens, interaction with co-medications, and enzyme gene polymorphisms on the dose–concentration–response relationship and, thus, on the given drug therapy. These models are particularly qualified to perform extrapolations beyond the available clinical data or the studied population group, which is critically important when extrapolating knowledge to clinically understudied groups such as children, where fewer data exist due to both ethical and technical constrains [5,12].

2.2. Application and Impact of PBPK Models on Individualized Drug Therapy

The role of PBPK models in individualized drug therapy is very promising but still maturating, particularly in the pediatric field. One of the major aspects in which PBPK models can contribute to individualized drug therapy is by forecasting the effects of genetic polymorphisms in key enzymes on *in vivo* drug exposure (AUC = area under the concentration time curve), *e.g.*, by incorporating the available *in vitro* metabolism data related to polymorphic enzyme allelic forms. Examples where PBPK models have been utilized include disposition of the antiviral oseltamivir in humans with CES1 activity that has been impaired by ethanol or loss-of-function genetic polymorphisms [13], the effect of CYP2C8 genotype on the anti-diabetic rosiglitazone exposure in the absence and presence of trimethoprim [14], the effect of CYP2D6 polymorphisms and multiple co-medications on the pharmacokinetics of the anti-psychotic agents aripiprazole, Iloperidone and risperidone, and the anti-muscarinic agent 5-hydroxymethyl tolterodine [15], or the effect of CYP3A5 genotype on tacrolimus [16–18]. It is important to note that these models were evaluated in adults and therefore this knowledge is still to be extrapolated to children, however, several publications have already shown the success achieved by scaling PBPK models from adults to children [19,20].

For patients with heart disease, the example of tacrolimus is of interest. Tacrolimus is an effective immunosuppressive for preventing rejection after solid-organ transplantation such as heart transplantation. Therapeutic drug monitoring (TDM) is mandatory to avoid toxic or sub-therapeutic concentrations. Tacrolimus is metabolized mainly by CYP3A4 and CYP3A5, and a polymorphic variation in CYP3A5 expression (expressors: individuals with at least one CYP3A5*1 allele, and non-expressors: individuals with CYP3A5*3/*3 mutant allele) results in different enzyme activity and therefore in different drug exposure. An integration of this genetic information in a PBPK model [18] resulted in a fair degree of agreement with observed tacrolimus concentrations (Fig. 2 and Table 1). Tacrolimus exposure (as AUC_{0-12}) was almost 2 times higher in CYP3A5 non-expressors than in expressors. A recent prospective study showed that when the CYP3A5 genotype of the patient is taken into consideration at the time of treatment initialization, a higher proportion of patients achieved the target trough concentration at an earlier time point and with fewer tacrolimus dose modifications [4] (Fig. 3).

For selected patients, application of such a model can further guide individual tacrolimus therapy in the context of the single CYP3A5 expressor or non-expressor genetic information. Predictions of these models can accompany therapeutic drug monitoring (TDM), enhance the adjustment of therapeutic doses and accelerate the time to reach the desired concentrations by an *a priori* exploration of drug levels on individual basis taking into consideration additional factors such as co-medications. Furthermore, the predicted total drug exposure can support or reject assumptions of non-compliance suspected by low concentrations measured during routine follow-up. These predictions can also minimize the need for intensively monitor drug levels in the first days of therapy initiation, which is the most critical phase, by using the measured trough concentration as a confirmatory rather than an exploratory finding. In stabilized patients, determination