The Role of Pharmacometabonomics in Predicting Drug Pharmacokinetics

Dorsa Varshavi, Dorna Varshavi and Jeremy R Everett*

Medway Metabonomics Research Group,

University of Greenwich,

Chatham Maritime,

Kent,

ME4 4TB

United Kingdom

* Author for Correspondence

Telephone: +44 (0)208 331 8323

Email: j.r.everett@greenwich.ac.uk

Abstract

Individual variability in drug response is a key challenge in current clinical practice and in drug discovery and development. Pharmacological response is closely associated with drug concentration at the site of action and therefore knowledge of drug pharmacokinetics is vital to delivering effective therapy. In addition to genetic polymorphisms, environmental factors also play an important role in determining drug efficacy, safety, metabolism and pharmacokinetics. The newly emerging field of pharmacometabonomics uses information from pre-dose metabolite profiles to predict individual drug responses, can be sensitive to both genetic and environmental factors and thus has great promise to help the future delivery of personalized medicine. This article introduces pharmacometabonomics and covers its application to the prediction of pharmacokinetics.

Editorial

Personalised or stratified therapy is a key goal for 21st century medicine in order to maximize therapeutic efficacy and minimize the likelihood of adverse drug reactions for groups of patients. In addition, personalised medicine has the potential to substantially enhance the process of drug discovery and development. [1] Thus far, personalized medicine has been mainly based on pharmacogenomics (PG), where an individual's genetic profile is used to

predict the clinical outcome of drug treatment.[2] There are now numerous PG studies associating human genetic polymorphisms with drug effects. The best-recognized examples are genetic polymorphisms of drug-metabolizing enzymes such as cytochrome P450 isoenzymes, *N*-acetyl transferases, sulfotransferases, and glucuronosyl-transferases.[3] Although genetic variation is a well-recognised factor contributing to drug response variability, the achievement of "individualized drug therapy" for a wide range of diseases is unlikely using genomic knowledge alone. This is because inter-individual variation in drug response is influenced by the complex interplay between genetic and environmental factors including nutritional status, lifestyle, age, gender, diseases, gut microbiota and co- or pre-administration of other drugs. These factors can significantly impact the pharmacokinetic (PK) characteristics of a drug including the processes of absorption, distribution, metabolism and excretion (ADME) and thereby cause inter-individual variation in drug effects.

In an alternative but complementary approach, pharmacometabonomics has now been applied to predict the efficacy, safety, metabolism and PK of drugs. Pharmacometabonomics is an extension of metabonomics [4] and is defined as 'the prediction of the outcome (for example, efficacy or toxicity) of a drug or xenobiotic intervention in an individual based on a mathematical model of pre-intervention metabolite signatures'. In a pharmacometabonomics study, the metabolite profiles in pre-dose biofluid samples (typically urine or blood plasma) from a group of subjects are analysed by technologies such as nuclear magnetic resonance (NMR) spectroscopy or mass-spectrometry (MS), the latter typically hyphenated with a separation technology. Multivariate statistical analysis methods, such as principal components analysis (PCA) or partial least squares (PLS), are then used to analyse the *pre-dose* metabolite profiles to discover statistically significantly different sub-groups of subjects whose post-dose outcomes, such as levels of efficacy or degree of drug metabolism, are different from those of other sub-groups.[5] Preferably, the predictive model is created on a training set of subjects and then tested in an independent, 'external' validation set.

Pharmacometabonomics was initially discovered in a study on a group of 75 Sprague-Dawley rats. It was shown that pre-dose urinary metabolite profiles could be used to predict the metabolism and the hepatotoxic effects of the analgesic paracetamol.[6] Subsequently, this approach was demonstrated in humans in a study of the metabolism of the same drug paracetamol [7] and a clear relationship was found between the volunteers' pre-dose, urinary, endogenous metabolite profiles and the post-dose metabolism of the drug. NMR-based

analysis revealed that human volunteers excreting relatively large amounts of the microbial co-metabolite *para*-cresol sulfate in their pre-dose urines tended to have a lower ratio of paracetamol sulphate to paracetamol glucuronide in their post-dose urines than individuals with low amounts of pre-dose urinary *para*-cresol sulfate.[7] *Para*-cresol-sulfate is a metabolite produced from the hepatic sulfonation of *para*-cresol, which itself is generated by gut bacteria, particularly *Chlostridium* species. Paracetamol and *para*-cresol have similar molecular structures and both compete for sulfation via the same human sulphotransferase enzymes, particularly SULT1A1. [8,9] This study demonstrated that the sulfonation of paracetamol (and potentially any other drug) can be restricted by competition from *para*-cresol, and also demonstrated the critical role of the gut microbiome in human drug metabolism.

Since its initial discovery,[6] pharmacometabonomics has been increasingly applied in both preclinical and clinical studies to predict drug safety, efficacy, metabolism and pharmacokinetics.[10] The potential of pharmaco-metabonomics in predicting the PK profile of a drug was first demonstrated in a study by Yoon et al. for the commonly used immunosuppressive drug, tacrolimus. [11] The efficacy of this drug is associated with a narrow therapeutic index combined with a large degree of variability in patient blood levels. Therefore, it is important to predict the PK of tacrolimus in order to minimise adverse drug reactions. Tacrolimus was administrated to 29 healthy, Korean males (75 ug/kg, oral) whilst controlling food intake and environmental conditions. Liquid chromatography-mass spectroscopy (LC-MS) analysis of the pre-dose urines of these volunteers resulted in detection of 1,256 metabolic ions, among which 42 key metabolic features were shown to be closely correlated with the drug's PK in terms of the area under the curve (AUC). Using LC-MS/MS along with database searching, 28 metabolites were identified and subsequently used to reconstruct a hypothetical metabolic network. To generate a more clinically applicable model, four metabolites (cortisol, acetyl-arginine, phosphoethanolamine, and 1-methylguanosine), with high contributions to the PLS model and representing four major metabolic pathways, were selected for predicting the AUC of tacrolimus. The new model successfully classified individuals into high, medium, and low AUC groups. This study demonstrated the potential of pharmacometabonomics to predict PK.

Kaddurah-Daouk et al used an LC-MS approach to show that pre-dose plasma levels of the bile acids chenodeoxycholic acid and deoxycholic acid were correlated to post-treatment

simvastatin levels in a broader study using pharmacometabonomics methodology to predict statin efficacy.[12]

Liu et al. applied pharmacometabonomics to predict the pharmacokinetic characteristics of triptolide in male Sprague−Dawley rats.[13] Triptolide is a major bioactive diterpenoid triepoxide that possesses a variety of anti-inflammatory, immunosuppressive and anti-tumor properties and has been used for centuries in traditional Chinese medicine for the treatment of immune-related diseases.[13] The clinical application of triptolide, however, is restricted by its narrow therapeutic index and high toxicity. Groups of rats were treated with one of three diets: normal, calorie-restricted or high fat diet, and then administered triptolide (0.60 or 1.80 mg/kg oral). Gas chromatography (GC)-MS analysis of the pre-dose serum detected 267 metabolite ions, of which 85 were identified. Multivariate regression analysis showed that the predose serum concentrations of creatinine and glutamate were linearly negatively correlated to postdose triptolide plasma maximal concentration (C_{max}) and AUC values.

The same group employed GC-MS analysis of pre-dose plasma to predict the pharmacokinetics of atorvastatin (oral, 20 mg/kg) in 48 healthy volunteers hospitalized at clinical research units with strict control over diet and environment.[14] Atorvastatin is an HMG-CoA reductase inhibitor that is generally used to lower levels of low-density lipoprotein cholesterol (LDL-C) in plasma and reduce the risk for coronary artery disease (CAD). The pharmacokinetics of atorvastatin vary considerably between individuals and hence its therapeutic efficacy is also variable.[15] The initial PLS multivariate analysis, conducted on 181 measured metabolite ions and 16 physiological and biochemical parameters from individuals in a training set (n = 36), revealed 63 and 57 variables which were highly correlated with atorvastatin C_{max}, and AUC respectively. Subsequently, sets of 17 and 12 key metabolites with high contributions to the initial PLS model and significant correlation to pharmacokinetic parameters, were selected to construct a refined model that could predict individualized C_{max} and AUC, respectively. This refined model allowed the prediction of the PK parameters of 12 other healthy volunteers in a validation set (with correlation coefficients of $r = 0.83$ for C_{max} and $r = 0.87$ for AUC) and could also successfully classify individual pharmacokinetic responses into subgroups.

The group of Barin-Le Guellec *et al* recently reported the use of GC-MS-based pharmacometabonomics to predict the clearance of methotrexate (MTX) in a cohort of 62

adult patients being treated for lymphoid malignancies.[16] Variable PK of MTX is known to be responsible for serious patient toxicities, even death, and over-exposure can occur even in the *same patient* between MTX treatment courses, thus indicating that genetic factors *per se* are not responsible for all of the variability observed.[16] In a well-designed study utilising internal and external validation of the models, the pre-dose urine levels of 28 metabolites were shown to be predictive of MTX clearance with mean prediction error and precision of 0.4% and 21% respectively. An orthogonal PLS discriminant analysis model showed a partial separation between patients with normal or delayed MTX elimination and whilst the specificity was excellent (93%), sensitivity was poor (42%) and model improvements would be required for clinical utility of this element. The model for the prediction of MTX clearance is however expected to have clinical utility and also gave insights into the underlying mechanisms of MTX excretion, including the role of organic anion transporters.[16]

Pharmacometabonomics, though still an emerging technology, has shown significant promise in the prediction of drug efficacy, safety and metabolism, in addition to the prediction of PK. Around 20 studies of pharmacometabonomics in humans have now been reported [17] in addition to pre-clinical studies. The major advantage of this technology is that it can inherently take into account both genetic and environmental influences in its predictive models. The existing studies clearly demonstrate the potential of pharmacometabonomics to help predict variation in pharmacokinetics and thereby to facilitate the delivery of personalised drug therapy. Further investigations will be required to demonstrate the broader utility of this approach for the general patient population and also to follow and predict optimal long term treatment.[18] It is clear that pharmacometabonomics is complementary to pharmacogenomics and therefore the integration of the two technologies will be both powerful [19] and could provide more insight into mechanisms underlying individual variation in drug response.

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