

Title: Mechanistic PBPK as an aid in identifying the size of covariate effects and design of POPPK studies: An example focusing on haematocrit as a determinant of tacrolimus clearance

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Objectives: Non-linear mixed effect modeling is often used for detecting the patient covariates which significantly influence the kinetics or dynamics of candidate drugs under development. The power of these studies to detect the covariate effects depends on a number of variables related to study design (overall sample size, size of the sub-proportion of individuals with certain covariates, and so on). Nonetheless, two main determinants of ability to identify covariates are the effect size and inherent variation of the measured entity. Assessing these, using mechanistic physiologically based pharmacokinetic (PBPK) models prior to carrying out population pharmacokinetic (POPPK) studies, would be of appreciable benefit in optimizing study design. Previously we have reported haematocrit (HC) as a co-variate for the dose-to-concentration (D/C) ratio of tacrolimus [1]. The aims of the present study were (1) to investigate the ability to simulate the effects of HC on D/C of tacrolimus using a mechanistic models of clearance implemented in a population-based pharmacokinetic simulator (Simcyp Ver 7, www.simcyp.com), and (2) to use clinical trial simulation in the assessment of statistical power of *in vivo* studies to detect the effects of cytochrome (CYP) 3A5 genotype and gender on the D/C ratio of tacrolimus in a Japanese liver transplant population.

Methods: *In vitro* pharmacokinetic parameters of tacrolimus including physicochemical characteristics and enzymatic metabolic kinetics were obtained from literature. Blood-to-plasma concentration ratio of tacrolimus was assumed to be 32.0 under HC value of 40% [1]. The D/C ratios in blood and plasma of six Japanese liver transplant subjects included in our previous report [1] were used for the analysis. Information on individual subject characteristics such as actual mean dose, age and body weight were incorporated into simulations to mimic the *in vivo* study design. These six patients were assumed to be poor metabolizer (PM) for CYP3A5. We also included one patient who was excluded from our previous analysis due an unusually high D/C value [1] (who was assumed to be an extensive metabolizer (EM) for CYP3A5), and simulated D/C ratios in blood and plasma in a similar way. Liver volume for Japanese liver transplant subjects was assumed to be 88% that of normal Japanese individuals [2]. Regarding intraindividual variation of CYP3A4 and CYP3A5 enzyme abundance, a coefficient of variation (CV) of 9.75% was applied based on the intraindividual variation of midazolam clearance reported by Kashuba *et al.* [3]. The statistical power to detect the effects of CYP3A5 genotype and gender was determined from 20 trials using a virtual Japanese liver transplant population selected from the Simcyp Simulator population database. For the gender study, PM for CYP3A5 of 58% was assumed [4]. CYP3A4 abundance in female Japanese was assumed to be 45.3% higher than that in male Japanese, based on the abundance ratio in male and female Caucasian. The ratio of male: female individuals in the population were 1:2 based on the gender distribution of liver transplant patients in Japan [5]. Tacrolimus dose was assumed to be 2.245 mg every 12 hr. To assess the power to detect differences due to gender, steady-state concentrations of tacrolimus in blood and plasma were simulated in individual Japanese virtual liver transplant subjects in studies of different sizes ($n = 6 - 600$). In order to investigate the effect of CYP3A5 genotype, studies containing 4 - 500 virtual subjects from the same population with EM:PM ratio of 1:1 were simulated. The power of the study was measured by the rate of detection of statistically significant differences in D/C ratio with the P value of less than 0.05 (two tail *t*-test).

Results: The simulated relationship between D/C values (in both blood and plasma) and HC corresponded well to those observed *in vivo* [1]. The difference between simulated D/C value and observed one for each patient was within twofold. Moreover, the correlation coefficient between blood D/C ratio and HC was larger than that between plasma D/C ratio and HC, which is also consistent with our previous finding [1]. The unusually high D/C values observed for one patient were, at least in part, explained by CYP3A5 genotype, although the observed

D/C value was still considerably, but less than twofold, higher than the simulated value. From the power studies, at a male:female ratio of 1:2, 600 or more subjects were required to detect statistically significant differences in the D/C value on tacrolimus between male and female ($p < 0.05$; two tail t -test) with 90% probability. For the detection of the effects of CYP3A5 genotype, 50 or more subjects were required to detect statistically significant differences in the D/C value of tacrolimus with 90% probability.

Conclusions: Using population-based pharmacokinetic simulations, it was possible to reproduce the relationship between D/C value of tacrolimus and HC value observed *in vivo* [1]. Although we did not examine different POPPK study designs as part of this investigation, the exercise could be used for examining efficiency of different designs. Therefore, mechanistic approach linking *in vitro* information on drug with knowledge of the system (as implemented in the *in vitro-in vivo* extrapolation [IVIVE] within Simcyp) is considered a useful tool for assessment of the influences of covariates on the pharmacokinetics. The example used in this investigation, *i.e.* tacrolimus, is a drug with extensive distribution into blood cells, and knowing HC variability in patients the study highlighted the importance of investigating adequate numbers of individuals in the detection of the effect of other patient characteristics such as CYP3A5 genotype or gender on D/C ratio. The liver transplant patients with the large variation in HC, that leads to increasing variability in D/C ratio, may not be an appropriate target population to analyze the influence of other patient characteristics on tacrolimus pharmacokinetics.

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