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# Enterohepatic Circulation Model for Population Pharmacokinetic Analysis

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**Abstract**

An enterohepatic circulation model based on physiological aspects of biliary excretion has been developed for population pharmacokinetic analysis. Mycophenolate mofetil was selected as a model drug for validation of the model. As a secondary objective, the model was used for pharmacokinetic comparison among different races.

The post-hoc plasma concentration–time course was well described by the newly developed enterohepatic model and a secondary peak arising from enterohepatic circulation was also well defined. The covariates predicted by the model agreed well with literature results.

The model is useful for evaluation of the covariates of an enterohepatically circulated drug. The population pharmacokinetic approach is of benefit for evaluating racial differences for a pharmacokinetic bridging package.

Enterohepatic circulation can affect terminal half-life, the area under the plasma concentration–time curve and bioavailability. Several pharmacokinetic models have been published for description of the pharmacokinetics of enterohepatically circulated drugs (Pederson & Miller 1980; Yamaoka et al 1990; Gabrielsson & Weiner 1997). The importance of population pharmacokinetics in drug development has increased recently because population pharmacokinetics, as part of the drug development programme, are thought to play a significant role and to provide information valuable for registration purposes (Vozeh et al 1996). Population pharmaco-kinetics can be used for ethnic comparisons and to provide a pharmacokinetic bridging package. Docetaxel has been well described in a bridging study with the purpose of internationally harmonizing clinical development (Tanigawara 1997). Since the agreement at the Fourth International Conference on Harmonization (Arey & Harron 1997), utilization of foreign clinical data has been greatly encouraged in Japan.

Except for the current model, no enterohepatic circulation model for population pharmacokinetics based on physiological aspects of biliary excretion has yet been reported. The author reported a prototype of the enterohepatic circulation model in 1997 (Funaki 1997). The aim of the current study was to establish this model for population pharmacokinetics and evaluate its usefulness in ethnic comparison of enterohepatically circulated drugs as a pharmacokinetic bridging package.

Mycophenolate mofetil, an immunosuppressant, was selected as a model drug because its pharmacokinetics and covariates are well defined (Bullingham et al 1998). Mycophenolate mofetil is a prodrug of mycophenolic acid, a glucuronidated immunosuppressant. Enterohepatic circulation of mycophenolic acid has been estimated to be 40% (approx.) with a range of 10 to 60% (Bullingham et al 1998). If similar results can be obtained in the current population pharmacokinetic analysis, this enterohepatic circulation model for population pharmacokinetics could be considered to be validated.

## Material and Methods

*Data*

The original data used for this analysis can be found elsewhere (Bullingham et al 1998) because

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the current analysis is a meta-analysis. Although a total of 140 subject data were used, the total number of plasma concentration–time courses was 270, because both steady-state and single dose data are included. The dosing range of mycophenolate mofetil was 100–2500 mg once or twice a day and doses were taken before or after a meal. Plasma samples were usually taken 0·5, 1, 2, 3, 4, 6, 8 and 12 h after dosing.

*Model*

The enterohepatic circulation model is shown in Figure 1. In contrast with another model (Gabrielsson & Weiner 1997), release of bile is assumed to occur as a bolus at the time of expulsion from the gall bladder ($t_{gap}$). After a single dose, the plasma concentration in the time window $t_{lag} \leq t < t_{gap}$, where $t_{lag}$ is the lag-time, can be described by equation 1:

$$C = \frac{k_a \cdot D}{(k_a - k) \cdot Vd} \left(e^{-k \cdot (t - t_{lag})} - e^{k_a \cdot (t - t_{lag})}\right) \quad (1)$$

where $k_a$ is absorption rate constant, k is elimination rate constant, D is dose and Vd is volume of distribution. When $t \geq t_{gap}$, the plasma concentration can be described by equation 2:

$$C = \frac{k_a \cdot D}{(k_a - k) \cdot Vd} \left(e^{-k \cdot (t - t_{lag})} - e^{k_a \cdot (t - t_{lag})}\right) + \frac{k_d \cdot D}{k} \left(1 + \frac{1}{(k_a - k)} \left(k \cdot e^{-k_a \cdot t_{gap}} - k_a \cdot e^{-k \cdot t_{gap}}\right)\right) \cdot \frac{k_a}{(k_a - k) \cdot Vd} \left(e^{-k \cdot (t - t_{gap})} - e^{-k_a \cdot (t - t_{gap})}\right) \quad (2)$$

After a multiple dose, the plasma concentration in the time window $t_{lag} \leq t < t_{gap}$ can be approximated by equation 3:

$$C = (C_{SS})_{min} \cdot e^{-k \cdot t} + \frac{k_a R \cdot D}{(k_a - k) \cdot Vd} \left(e^{-k(t - t_{lag})} - e^{-k_a (t - t_{lag})}\right) \quad (3)$$

where $(C_{SS})_{min}$ is the minimum concentration at steady-state and R the accumulation factor. R has been defined by Gibaldi & Perrier (1982) but is more complicated in this work; to simplify the model R is defined as:

$$R = \frac{1}{(1 - e^{-k \cdot \tau})} \quad (4)$$

where $\tau$ is the dosing interval. When $t \geq t_{gap}$ the plasma concentration after a multiple dose can be approximated by:

$$C = (C_{SS})_{min} e^{-kt} + \frac{k_a \cdot R \cdot D}{(k_a - k) Vd} \left(e^{-k \cdot (t - t_{lag})} - e^{-k_a \cdot (t - t_{lag})}\right) + \frac{k_d \cdot R \cdot D}{k} \left(1 + \frac{1}{(k_a - k)} \left(k \cdot e^{-k_a \cdot t_{gap}} - k_a \cdot e^{-k \cdot t_{gap}}\right)\right) \cdot \frac{k_a}{(k_a - k) \cdot Vd} \left(e^{-k \cdot (t - t_{gap})} - e^{-k_a \cdot (t - t_{gap})}\right) \quad (5)$$

*Software*

NONMEM V (NONMEM Project Group 1998) was used for population pharmacokinetic analysis. Visual Fortran 5.0 (Digital Equipment Corporation 1997) was used as the Fortran compiler.

*Covariates*

Covariate candidates were screened by univariate analysis, by use of NONMEM. After the analysis, covariates were incorporated into the model one by one (stepwise forward inclusion). When object function was reduced by more than 10·83 after incorporating a new covariate, the covariate was kept in the model and incorporation of another was attempted. After development of a full model, its stability was checked by subtracting each covariate individually (backward elimination). Where object function was reduced by more than 3·84 after subtracting the covariate, the subtracted covariate was put back into the model.

## Results and Discussion

*Demographic and background data*

Demographic data from patients used for the population pharmacokinetic analysis are shown in Table 1. Japanese, Caucasian, African American and other races were categorized. The balance in sample size might influence evaluation of intergroup differences (Tanigawara & Hori 1991). The total number of Japanese and Caucasian patients was nearly equal; the mean body weight of Japanese was slightly less than that of Caucasians. Background data from patients is shown in Table 2. Feeding habits during drug intake were substantially different for Japanese and Caucasians. Most Japanese (84·7%) took the drug after a meal whereas most Caucasians (82·9%) took it before. Although AUCs of mycophenolic acid were statistically equivalent in the fed and fasted states, the

Table 1. Demographics of patients used for population-pharmacokinetic analysis.

| | Number of patients | Age (years) | Weight (kg) | Creatine clearance ($mL\,min^{-1}$) |
|---|---|---|---|---|
| Japanese | 58 | 45 ± 13 (17–66) | 54·5 ± 11·4 (35·0–100·9) | 51·5 ± 27·0 (5·0–101·8) |
| Caucasian | 68 | 54 ± 12 (20–77) | 74·7 ± 18·0 (38·2–127·0) | 58·6 ± 25·6 (4·3–125·0) |
| African American | 9 | 41 ± 12 (18–57) | 73·1 ± 18·7 (49·5–102·0) | 30·3 ± 27·2 (6·8–81·9) |
| Others | 5 | 53 ± 8 (44–61) | 81·4 ± 2·7 (78·0–84·0) | 93·8 ± 25·8 (48·5–111·0) |
| Total | 140 | 49 ± 13 (17–77) | 66·5 ± 183 (35·0–127·0) | 55·1 ± 27·9 (4·3–125·0) |

Results are means ± s.d. (minimum–maximum). Immediate post-transplant data were excluded from the analysis.

Table 2. Background of patients used for population-pharmacokinetic analysis.

| | Frequency (%) | | | | |
|---|---|---|---|---|---|
| | Japanese (n = 58) | Caucasian (n = 68) | American African (n = 9) | Others (n = 5) | Total (n = 140) |
| Gender | | | | | |
| Male | 56·9 | 48·5 | 66·7 | 60·0 | 53·6 |
| Female | 43·1 | 51·5 | 33·3 | 40·0 | 46·4 |
| Disease | | | | | |
| Rheumatoid arthritis | 31·0 | 39·7 | 22·2 | 80·0 | 36·4 |
| Renal transplant | 69·0 (n = 118)[a] | 60·3 (n = 123)[a] | 77·8 (n = 18)[a] | 20·0 (n = 11)[a] | 63·6 (n = 270)[a] |
| Feeding habits | | | | | |
| Fasted | 0·0 | 11·4 | 11·1 | 36·4 | 7·4 |
| After meal | 84·7 | 5·7 | 5·6 | 18·2 | 40·7 |
| Before meal | 15·3 | 82·9 | 83·3 | 45·5 | 51·9 |
| Maalox | | | | | |
| No | 30·5 | 11·4 | 11·1 | 36·4 | 20·7 |
| Yes or unknown | 69·5 | 88·6 | 88·9 | 63·6 | 79·3 |

[a]Number of plasma–concentration profiles.

median $t_{max}$ was higher and the mean $C_{max}$ was statistically significantly lower in the fed state than in the fasting state (Bullingham et al 1998). Therefore, bias in feeding habits for Japanese and Caucasians might complicate the covariate of race if both race and feeding habits are covariate.

*Pharmacokinetic model*

As shown in Figure 1, to simplify the model mycophenolic acid was assumed to be a recirculating entity in this study. In the real situation mycophenolic acid is glucuronidated in the liver and excreted in the bile as glucuronidated mycophenolic acid. Glucuronidated mycophenolic acid excreted in the bile is rehydrolysed to mycophenolic acid in the intestine and reabsorbed as mycophenolic acid. The de-esterification of mycophenolate mofetil was also not included in the model.

The steady-state equation was approximated in this study because the analytical equation for steady-state was difficult to obtain. The error in the approximation can be evaluated by simulated multiple-dose administration. Simulation results show that the error of approximation is marginal (not shown).

Typical plasma concentration–time courses are shown in Figure 2. The post-hoc estimate by NONMEM agreed well with the observed value. The secondary peak arising from enterohepatic circulation was also well defined. The clearance (CL) values in this study were $25–46\,L\,h^{-1}$, in good agreement with the previously reported value for the hepatic clearance of mycophenolic acid (Bullingham et al 1998). These partially validate the current model of enterohepatic circulation.

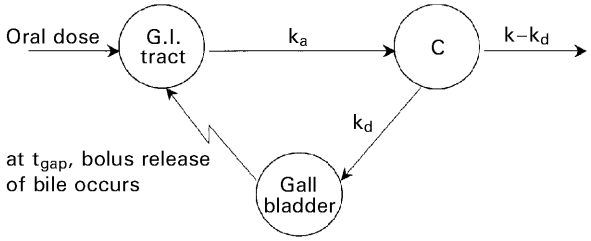


Figure 1. The enterohepatic circulation model used for population pharmacokinetic analysis: $k_a$, absorption rate constant; $k_d$, excretion rate constant into gallbladder; k, elimination rate constant; $t_{gap}$, expulsion time of gallbladder.

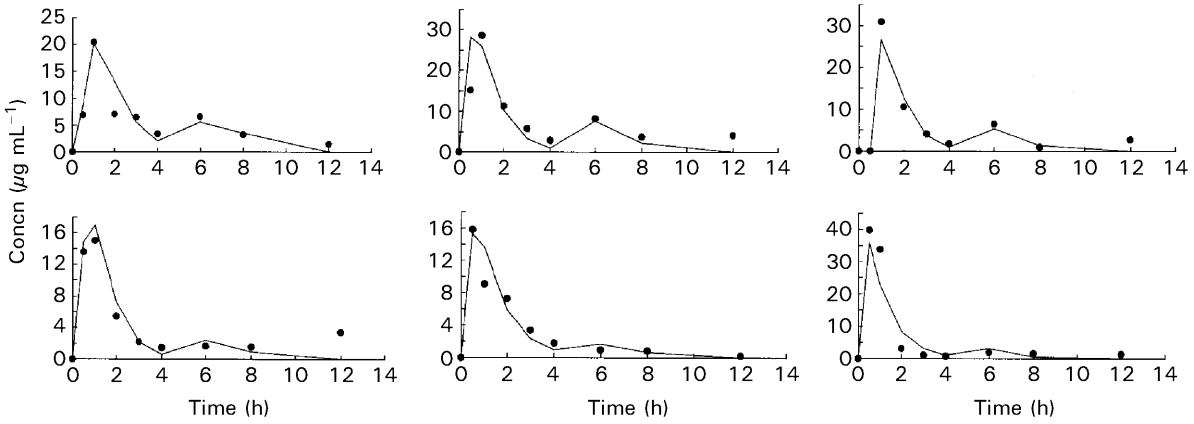


Figure 2. Comparison between values calculated by NONMEM (solid line) and the values observed for typical patients.

Table 3. Covariates in univariate analysis, found by use of NONMEM.

| Parameter | Covariates |
|---|---|
| $k_a$ | Food, race |
| k | Food, race |
| Vd | Food, race, weight, Maalox, creatinine clearance |
| $t_{lag}$ | Maalox |

Gender and disease were not found as covariates for any parameters. $k_a$ and k are the absorption and elimination rate constants, Vd is the volume of distribution, and $t_{lag}$ is the lag-time.

This model assumes only one gall-bladder discharge in the given sampling schedule; under physiological conditions several discharges of the gallbladder might occur. However, such modelling is very difficult for a limited number of samples, especially for population pharmacokinetic analysis.

*Covariates*

The covariates which were found in this study are shown in Table 3. Both feeding habits and race were found as covariates for $k_a$, k and Vd. As described previously, there is bias in feeding habits for Japanese and Caucasians and this might complicate the results. A comparison of our results with published data (Bullingham et al 1998) is shown in Table 4; they did not contradict the previous results. Baseline background data, i.e. pre-dose data on day 1, were used in this study as covariates even in the steady state. Therefore, identification of a

Table 4. Comparison of current and previous results.

| Current results | Previous results (Bullingham et al 1998) |
|---|---|
| Feeding habits affected $k_a$. The absorption rate constant in the fed state was smaller than in the fasted state | $AUC_{24}$ was statistically equivalent in the fed and fasted states; $t_{max}$ was delayed and $C_{max}$ was lower in the fed state |
| Maalox had no effect on $k_a$ | Maalox reduced $AUC_{24}$ and $C_{max}$ whereas $t_{max}$ was unchanged |
| The clearance derived by the model was 25–46 L h$^{-1}$ | The hepatic clearance was ca 24 L h$^{-1}$. |
| Bodyweight and creatinine clearance were found as covariates for Vd | Age, creatinine clearance and body weight were found have important effects on the AUC for healthy subjects<br>Creatinine clearance had minimal effect on AUC in renal transplant patients. |
| Racial effect was found on k and Vd, thus CL was ca 25 L h$^{-1}$ for Japanese and ca 45 L h$^{-1}$ for Caucasians | The Japanese population seemed to have a systemically higher $AUC_{12}$ than Caucasians. This apparent racial difference might have been complicated by differences in body weight |
| Gender and age were not found as covariates for any pharmacokinetic parameters | Gender had no effect on the pharmacokinetics, although occasionally $C_{max}$ and AUC were slightly higher for women than for men |

$k_a$ and k are the absorption and elimination rate constants, Vd is the volume of distribution, and CL is the clearance. $AUC_{12}$ and $AUC_{24}$ are the areas under the plasma concentration–time curves between 0 and 12 and 0 and 24 h, respectively. $C_{max}$ is the maximum plasma concentration and $t_{max}$ is the time at which $C_{max}$ is reached.

Table 5. Comparison of mean pharmacokinetic parameters among races.

| | $k_a$ ($h^{-1}$) | k ($h^{-1}$) | Vd (L) | | $t_{lag}$ (h) |
|---|---|---|---|---|---|
| | | | No Maalox | Maalox[a] | |
| Fasted | | | | | |
| Japanese | 1·22 | 0·859 | 31·6 | 43·0 | |
| Caucasian | 1·43 | 1·062 | 43·1 | 58·6 | |
| African American | 2·33 | 0·508 | 83·1 | 113·0 | |
| Others | 2·86 | 0·767 | 49·4 | 67·1 | |
| After meal | | | | | |
| Japanese | 0·692 | 0·692 | 35·8 | 48·6 | |
| Caucasian | 0·856 | 0·856 | 48·7 | 66·2 | |
| African American | 0·409 | 0·409 | 93·9 | 127·7 | |
| Others | 0·618 | 0·618 | 55·8 | 75·8 | |
| Before meal | | | | | |
| Japanese | 2·72 | 0·458 | 54·1 | 73·6 | |
| Caucasian | 2·90 | 0·566 | 73·7 | 100·2 | |
| African American | 11·83 | 0·271 | 142·1 | 193·3 | |
| Others | 13·70 | 0·409 | 84·4 | 114·8 | |
| No Maalox | | | | | 0·412 |
| Unknown Maalox[a] | | | | | 0·274 |
| IS variation (%) | 252 | 27·6 | 38·9 | | 70·7 |

[a]It was unknown whether or not Maalox was taken. $k_a$ and k are the absorption and elimination rate constants, Vd is the volume of distribution, and $t_{lag}$ is the lag-time.

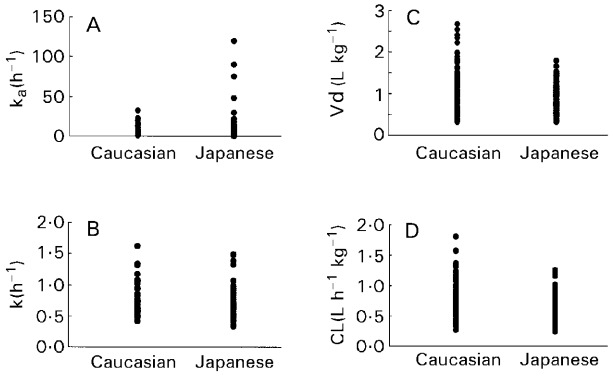


Figure 3. Comparison of pharmacokinetic parameters for Caucasian and Japanese patients. A. Absorption rate constant, B. elimination rate constant, C. volume of distribution, D. clearance.

time-dependent covariate might be necessary in the future to describe covariates in more detail.

*Ethnic comparison*

A comparison of pharmacokinetic parameters found in covariates is shown in Table 5. The difference between each pharmacokinetic parameter for Japanese and Caucasians is not significant if inter-subject variation is taken into account. In contrast, the difference between African Americans and Caucasians is large. The reason race was found to be a covariate can be partially explained by the difference between African Americans and Caucasians. Comparison of the pharmacokinetic parameters $k_a$, k, Vd and CL is shown in Figure 3. Some outliers can be observed in $k_a$. Excluding the outliers, $k_a$ were largely similar for Japanese and Caucasians. The other parameters of k, Vd and CL were also almost equal for the two groups.

Comparison of simulated plasma concentrations for Japanese and Caucasian virtual patients is shown in Figure 4, where the same age, weight and creatinine clearance are assumed. From these results the pharmacokinetics of mycophenolic acid can be concluded to be similar for these two groups.

*Conclusion*

The current enterohepatic circulation model is useful for evaluation of the covariates of an enterohepatically circulated drug. The population pharmacokinetic approach is of benefit for evaluating racial differences for a pharmacokinetic bridging package.

## References

Arey, P. F. D., Harron, D. W. G. (eds) (1997) Proc. Third Int. Conf. Harmonization, pp 321–414

Bullingham, R. E. S., Nicholls, A. J., Kamm, B. R. (1998) Clinical pharmacokinetics of mycophenolate mofetil. Clin. Pharmacokinet. 34: 429–455

Digital Equipment Corporation (1997) DIGITAL Fortran Language Reference Manual, Maynard, MA

Funaki, T. (1997) The 117th Annu. Meeting Pharm. Soc. Jpn

Gabrielsson, J., Weiner, D. (1997) Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications, 2nd edn, Kristianstads Boktryckeri, Sweden, pp 546–550

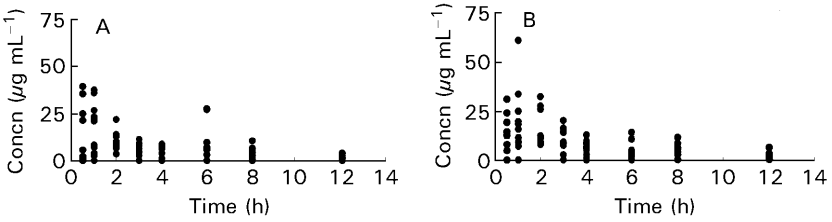


Figure 4. Comparison of simulated plasma concentrations for Caucasian (A) and Japanese (B) virtual rheumatoid arthritis patients, assuming age 45 years, weight 50 kg and clearance 60 mL $min^{-1}$. Each racial group comprised 12 subjects. Sigma of 8·3 and omegas of 6·37 for $k_a$, 0·0763 for k, 0·151 for Vd, 0·00036 for $t_{gap}$, 0·9 for $k_d$/k and 0·5 for $t_{lag}$ were used for the simulation.

Gibaldi, M., Perrier, D. (1982) Pharmacokinetics, 2nd edn, Marcel Dekker, New York, p. 121

NONMEM Project Group (1998) NONMEM Users Guide, University of California at San Francisco

Pederson, P. V., Miller, R. (1980) Pharmacokinetics and bioavailability of cimetidine in humans. J. Pharm. Sci. 69: 394–398

Tanigawara, Y. (1997) In: Aarons, L., Balant, L. P., Danhof, M., Gex-Fabry, M., Gundert-Remy, U. A., Karlsson, M. O., Mentre, F., Morselli, P. L., Rombount, F., Rowland, M., Steimer, J.-L., Vozeh, S. (eds) European Cooperation in the Field of Scientific and Technical Research, European Communities, Belgium, pp 25–37

Tanigawara, Y., Hori, R. (1991) In: Rowland, M., Aarons, L. (eds) European Cooperation in the Field of Scientific and Technical Research, European Communities, Belgium, pp 223–232

Vozeh, S., Steimer, J. L., Rowland, M., Morselli, P., Mentre, F., Balant, L. P., Aarons, L. (1996) The use of population pharmacokinetics in drug development. Clin. Pharmacokinet. 30: 81–93

Yamaoka, K., Kanba, M., Toyoda, Y., Yano, Y., Nakagawa, T. (1990) Analysis of enterohepatic circulation of cefixime in rat by Fast Inverse Laplace Transform (FILT). J. Pharmacokinet. Biopharm. 18: 545–559