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Validation of a semi-physiological model for caffeine in healthy subjects and cirrhotic patients

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ABSTRACT

The objective of this paper was to validate a previously developed semi physiological model to simulate bioequivalence trials of drug products. The aim of the model was to ascertain whether the measurement of the metabolite concentration–time profiles would provide any additional information in bioequivalence studies (Fernandez-Teruel et al., 2009a,b; Navarro-Fontestad et al., 2010).

The semi-physiological model implemented in NONMEM VI was used to simulate caffeine and its main metabolite plasma levels using caffeine parameters from bibliography. Data from 3 bioequivalence studies in healthy subjects at 3 different doses (100, 175 and 400 mg of caffeine) and one study in cirrhotic patients (200 or 250 mg) were used. The first aim was to adapt the previous semi-physiological model for caffeine, showing the hepatic metabolism with one main metabolite, paraxanthine. The second aim was to validate the model by comparison of the simulated plasma levels of parent drug and metabolite to the experimental data.

The simulations have shown that the proposed semi-physiological model was able to reproduce adequately the pharmacokinetic behavior of caffeine and paraxanthine in both healthy subjects and cirrhotic patients at all the assayed doses. Therefore, the model could be used to simulate plasma concentrations *vs.* time of drugs with the same pharmacokinetic scheme as caffeine, as long as their population parameters are known, and it could be useful for bioequivalence trial simulation of drugs that undergo hepatic metabolism with a single main metabolite.

1. Introduction

Different population semi-physiological models have been employed in previous studies (Fernandez-Teruel et al., 2009a,b; Navarro-Fontestad et al., 2010) to determine whether the measurement of the concentration–time profiles of metabolites would provide any relevant information on the biopharmaceutical performance of the products under comparison in bioequivalence studies, since FDA and EMA bioequivalence guidelines differed with regard to metabolite requirements (EMA, 2010; FDA, 2003). In principle, the evaluation of bioequivalence should be based on parent drug concentrations because the concentration–time profile of the parent drug is more sensitive to changes in the biopharmaceutical performance than that of a metabolite. However in certain cases the measurement of the metabolites is also necessary (e.g. for the FDA when the metabolite is active and it is formed pre-systemically, and for the EMA it was necessary when the pharmacokinetics is non-linear). In other cases, where the parent drug concentrations are difficult to measure due to its rapid elimination or instability, the metabolite is used as a substitute of the

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parent drug. Therefore in these cases, it is necessary to verify that the metabolite is sensitive to changes in the biopharmaceutical performance and able to discriminate between bioequivalence and non-bioequivalent products based on the parent drug exposure, especially if activity resides in the parent drug.

In order to explore parent drug and metabolite suitability for bioequivalence comparisons several semi-physiological models were designed and implemented in NONMEM to perform bioequivalence trials simulations and to establish the more sensitive analyte to detect drug product differences (Fernandez-Teruel et al., 2009a,b; Navarro-Fontestad et al., 2010).

The next step was to validate the proposed semi-physiological model, comparing the results of NONMEM simulations with published *in vivo* results in humans. The present paper shows the application of the previously designed models to caffeine, a well-known substance with hepatic metabolism and one main metabolite. The aims of this study were (1) to adapt the semi-physiological model (with hepatic metabolism and one main metabolite) implemented in NONMEM to caffeine and (2) to validate the model by comparing the simulated plasma concentrations of caffeine and its main metabolite with experimental caffeine data in healthy subjects and cirrhotic patients.

2. Material and methods

2.1. Caffeine pharmacokinetics

Caffeine gastrointestinal absorption is rapid and complete. In man, 99% of the administered dose was absorbed in 45 min, mainly from the small intestine but also 20% from the stomach (Fredholm, 2010). It is widely distributed in the total body water and it is eliminated by apparent first-order kinetics. There is minimal or no first-pass metabolism (Fredholm, 2010; McLean and Graham, 2002).

In vivo and *in vitro* studies have demonstrated that caffeine is metabolized mainly via N-3 demethylation to paraxanthine (Begas et al., 2007). Caffeine is metabolized in the liver by the cytochrome P-450 enzyme system. In human adults, more than 80% of caffeine is biotransformed to paraxanthine (Fredholm, 2010; McLean and Graham, 2002). Theobromine, theophylline and 1,3,7-trimethyluric acid are other metabolites (Kot and Daniel, 2008), being the kidney the main organ responsible for their elimination (Fredholm, 2010; McLean and Graham, 2002).

2.2. Description of the proposed model

A semi-physiological model (Fig. 1) was used to represent caffeine pharmacokinetics, with the following compartments: caffeine dissolved in lumen, caffeine in enterocytes, caffeine in liver, caffeine in the central compartment and paraxanthine (main caffeine metabolite) in the central compartment. Other metabolites were not included in the model, but 80% paraxanthine formation was considered.

The dose was administered as an oral solution of caffeine in a single dose scheme. Caffeine in the lumen is absorbed into enterocytes within a fixed operative absorption time (OAT) (Mudie et al., 2010). Once absorbed, caffeine reaches the systemic circulation after passing through the liver. Caffeine is rapidly distributed in one compartment and it is eliminated by hepatic metabolism. Paraxanthine is formed in the liver and it is distributed in one compartment and eliminated by renal excretion.

The equations that represent the amount change of caffeine and paraxanthine over time in each compartment are:

- The rate of change of caffeine amount in lumen depends on the absorption into the enterocytes:

$$\frac{dQ}{dt} = -k_{Aap} \cdot Q_L \tag{1}$$

where k_{Aap} is the apparent first order absorption rate constant of caffeine and Q_L is the drug amount dissolved in lumen.
Caffeine absorption takes place only during a fixed time (Mudie et al., 2010), called operative absorption time (OAT). The apparent absorption rate constant is defined by the following equation:

$$k_{Aap} = k_A \cdot \left(1 - \frac{t^h}{OAT^h + t^h}\right) \tag{2}$$

where k_A is the *true* absorption rate constant, t is the time after administration and h is the Hill constant, the shape parameter that makes the apparent absorption rat constant, k_{Aap} being close to k_A when time is less than OAT, and being zero when time exceeds the OAT.

- The rate of change of the amount of caffeine in the enterocytes (QE) depends on the absorption process, the caffeine coming from the central compartment, and the caffeine exit to the portal vein:

$$\frac{dQ}{dt} = k_{Aap} \cdot Q_L + \phi_E \cdot C_C - \phi_E \cdot C_E \tag{3}$$

where ϕ_E is the enteric blood flow, C_C is the caffeine concentration in the central compartment and C_E is the caffeine concentration in the enterocytes.

- The time course of the amount of caffeine in the liver (Q_H) depends on the input of caffeine coming from the portal vein and central compartment, and the exit to the central compartment in two fractions: as paraxanthine after caffeine metabolism and as unchanged caffeine. Caffeine is metabolized to form paraxanthine and the caffeine fraction escaping the hepatic metabolism returns to the central compartment as unchanged caffeine:

$$\frac{dQ}{dt} = \phi_E \cdot C_E + \phi_H \cdot C_C - (\phi_H + \phi_E) \cdot E_H \cdot C_H - (\phi_H + \phi_E) \cdot (1 - E_H) \cdot C_H \tag{4}$$

where ϕ_H is the hepatic blood flow, E_H is the hepatic extraction ratio and C_H corresponds to the caffeine concentration in the liver.

- Similarly, the rate of change of the caffeine amount in the central compartment (Q_C) is governed by the fraction of caffeine escaping metabolism in liver (which reaches the systemic circulation with a blood flow that sums gut and liver blood flows) and the exit of caffeine to enterocytes and liver:

$$\frac{dQ_C}{dt} = (1 - E_H) \cdot (\phi_H + \phi_E) \cdot C_H - \phi_E \cdot C_C - \phi_H \cdot C_C \tag{5}$$

- The rate of change of the amount of the metabolite paraxanthine in the central compartment (Q_{CPx}) depends on the rate of paraxanthine formation as a result of the caffeine metabolism in liver (considering that the 80% of the caffeine metabolism results in paraxanthine (Fredholm, 2010; McLean and Graham, 2002)) and the paraxanthine elimination rate in urine:

$$\frac{dQ_{CPx}}{dt} = 0.8 \cdot E_H \cdot (\phi_H + \phi_E) \cdot C_H - k_{elPx} \cdot Q_{cPx} \tag{6}$$

where C_{CPx} is the paraxanthine concentration in the central compartment, k_{elPx} is the paraxanthine first order elimination rate constant of elimination.

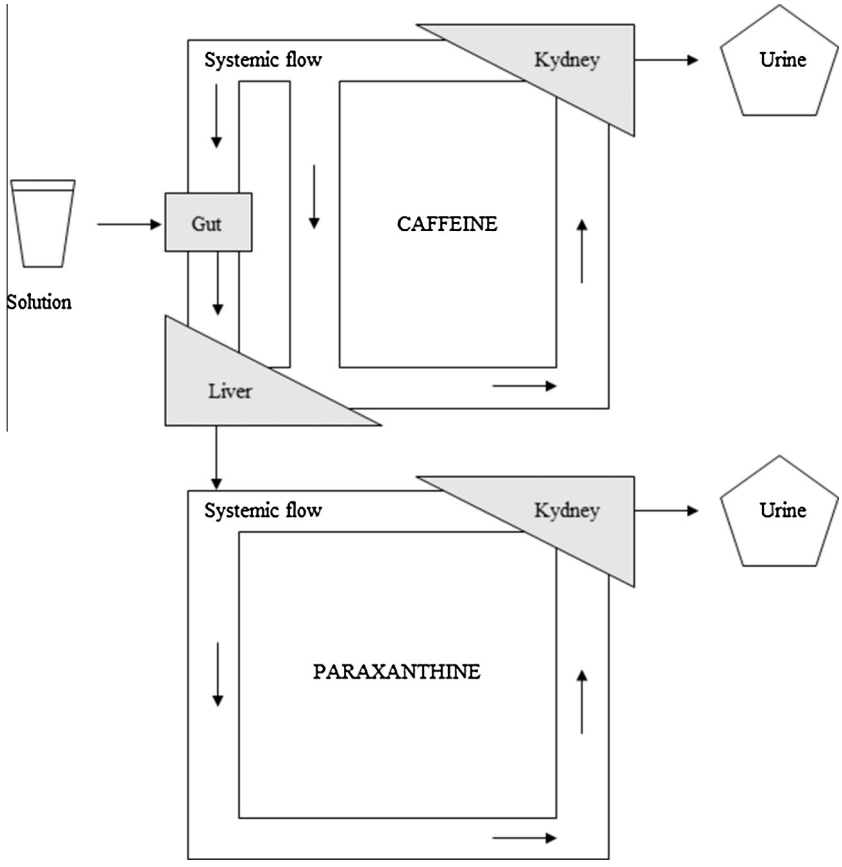

Fig. 1. Proposed semi-physiological model used to represent the caffeine LADME process.

The value of E_H depends on the liver intrinsic clearance (CL^i_{CH}) which is reduced as the C_H increases (Labaune, 1988; Ritschel and Kearns, 2004) and the hepatic and enteric flows:

$$E_H = \frac{CL^i_{C_H}}{\phi_H + \phi_E + CL^i_{C_H}} \quad CL^i_{C_H} = \frac{CL^i_{0_H} \cdot K_{M_H}}{K_{M_H} + C_H} \tag{7}$$

where CL^i_{0H} is the caffeine hepatic intrinsic clearance corresponding to linear (concentration independent) conditions and K_{MH} is the caffeine hepatic Michaelis–Menten constant.

The concentrations (C) of caffeine and paraxanthine in the different compartments are calculated as the ratio between the amount (Q) and the compartment volume (V).

The plasma protein binding fraction was not considered, in order to simplify the model because caffeine is a drug with low protein binding (Musteata et al., 2006). The unbound fraction was assumed to be equal to 1 and independent of the drug concentration.

2.3. Dose and basal caffeine

The simulations were performed with the same administered doses as in the reference articles that were used to validate the model. For simulations in healthy subjects, three oral dose levels were used: 100, 175, and 400 mg of caffeine base. Basal plasma concentrations measured in each reference article were also considered. Plasma concentrations at the pre-dose sample obtained from the plasma concentration–time curves were transformed into amount and were added to the dose. Consequently, the dose of 100 mg was transformed into 110 mg (10 mg correspond to basal caffeine (Turpault et al., 2009)), the dose of 175 mg was transformed into 219 mg (44 mg correspond to basal caffeine (Haller et al., 2002)) and the dose of 400 mg was maintained at 400 mg, as the exclusion criteria in the reference study were more restrictive regarding caffeine ingestion (McLean and Graham, 2002). For cirrhotic patients, the experimental data were taken from 12 cirrhotic patients that had received a first intravenous bolus of 100.6 mg of caffeine base and a second oral dose (24 h) of 200 or 250 mg of caffeine base, depending on the subject weight. This study was simulated considering the experimental intravenous administration rate and the basal plasma levels of caffeine for each individual subject, because the individual data are described in the original publication (Marco Carbayo, 2008).

2.4. Population parameter values

Population parameter values used in the simulations (Table 1) were obtained from published experimental data. Physiologic parameters were the same in both data sets (healthy subjects and patients) except hepatic artery and portal vein blood flows, which are altered in cirrhotic patients. Michaelis–Menten constant (K_M) of caffeine was also considered to be the same in both groups. Pharmacokinetic parameters were different in each simulation (healthy subjects vs. patients) because they were taken from each reference paper including different populations (Haller et al., 2002; Marco Carbayo, 2008; McLean and Graham, 2002; Turpault et al., 2009).

Michaelis–Menten constant (K_M) of caffeine was obtained using published K_M values of the metabolism of caffeine to form paraxanthine, theobromine and theophylline: a weighted average of the 3 K_M values was obtained, considering the formation percentage of each metabolite (Ha et al., 1996; McLean and Graham, 2002). The volume of the liver compartment was obtained from

Table 1
Parameters. Data in italics are physiologic parameters.

Parameters	Value (units)
Common parameters	
Michaelis–Menten constant (K_M) of caffeine in liver	0.19 (mmol/L)
Liver compartment volume (V_H)	*1.1 (L)*
Enterocytes compartment volume (V_E)	*1.8 (L)*
Operative absorption time (OAT)	*3.5 (h)*
Hill coefficient (h)	*20*
Parameters for dose of 100 mg	
Hepatic artery blood flow (ϕ_H	*18 (L/h)*
Enteric blood flow (ϕ_E	*72 (L/h)*
Caffeine hepatic intrinsic clearance at time 0 (CL_0^i)	5.5 (L/h)
Real absorption constant (k_A)	8.5 (h^{-1})
Paraxanthine elimination constant (k_{elPx})	0.125 (h^{-1})
Caffeine central compartment volume (V_C)	40 (L)
Paraxanthine central compartment volume (V_{Px})	40 (L)
Parameters for dose of 175 mg	
Hepatic artery blood flow (ϕ_H	*18 (L/h)*
Enteric blood flow (ϕ_E	*72 (L/h)*
Caffeine hepatic intrinsic clearance at time 0 (CL_0^i)	3.5 (L/h)
Real absorption constant (k_A)	1.95 (h^{-1})
Paraxanthine elimination constant (k_{elPx})	0.110 (h^{-1})
Caffeine central compartment volume (V_C)	30.8 (L)
Paraxanthine central compartment volume (V_{Px})	30.8 (L)
Parameters for dose of 400 mg	
Hepatic artery blood flow (ϕ_H	*18 (L/h)*
Enteric blood flow (ϕ_E	*72 (L/h)*
Caffeine hepatic intrinsic clearance at time 0 (CL_0^i)	5 (L/h)
Real absorption constant (k_A)	1.55 (h^{-1})
Paraxanthine elimination constant (k_{elPx})	0.108 (h^{-1})
Caffeine central compartment volume (V_C)	44 (L)
Paraxanthine central compartment volume (V_{Px})	44 (L)
Parameters for cirrhotic patients	
Hepatic artery blood flow (ϕ_H	*40 (L/h)*
Enteric blood flow (ϕ_E	*24 (L/h)*
Caffeine hepatic intrinsic clearance at time 0 (CL_{0H}^i)	0.645 (L/h)
Real absorption constant (k_A)	0.61 (h^{-1})
Paraxanthine elimination constant (k_{elPx})	0.0156 (h^{-1})
Caffeine central compartment volume (V_C)	45.9 (L)
Paraxanthine central compartment volume (V_{Px})	45.9 (L)

the liver volume (Heinemann et al., 1999), assuming a 70% of water content. The volume of the enterocyte compartment was obtained considering the villi absorption surface and the intestinal cellular compartment volume (Gonzalez-Alvarez et al., 2005). The operative absorption time (OAT) and the Hill coefficient were set to constrain the absorption process during the 3.5 first hours after the administration (Mudie et al., 2010). The hepatic blood flow represents the hepatic artery blood flow and the intestinal blood flow represents the portal vein blood flow. For healthy subjects, the total hepatic blood flow was set to 90 L/h, being 20% the hepatic artery blood flow and 80% the portal vein blood flow (Bradley et al., 1945; Carlisle et al., 1992). For cirrhotic patients, the hepatic artery and portal vein blood flow were set to 40 and 24 L/h respectively (Bradley et al., 1952; Moreno et al., 1967). The intrinsic clearances ($CL^i{}_0$) were obtained from the total metabolic clearance (CL) reflected in each reference article (Haller et al., 2002; Marco Carbayo, 2008; McLean and Graham, 2002; Turpault et al., 2009) and the total blood flow (ϕ) using the following equation:

$$CL = \frac{CL_0^i \cdot \phi}{CL_0^i + \phi} \tag{8}$$

This approximation could lead to an underestimation of $CL^i{}_0$ if the system is under non-linear conditions. Nevertheless the *CL* reported in the references corresponded to plasma levels clearly below the K_M value.

The *real* absorption rate constant was obtained considering the elimination half-life and the time of the maximum plasma concentration (Labaune, 1988; Ritschel and Kearns, 2004) shown in each publication. The elimination rate constant of paraxanthine was obtained from clearance and volumes of distribution, assuming its clearance is similar to that of caffeine. The caffeine and paraxanthine central volumes were assumed to be the same (Haller et al., 2002; Marco Carbayo, 2008; McLean and Graham, 2002; Turpault et al., 2009).

Table 2
Variability models and magnitude of the variability used in the simulations.

Inter-individual variability model		
$\theta_i = \theta_p \cdot e^{\eta_i}$	$\eta_i = N(0, \omega_i)$	$\omega_i = 0.04$
Residual variability model		
$y_i = y_p \cdot e^{\varepsilon_i}$	$\varepsilon_i = N(0, \sigma_i)$	$\sigma_i = 0.04$

2.5. Individual parameters

The individual parameters are the result of applying the inter-individual and residual variability models of Table 2 to population parameters of Table 1. The η and ε represent the set of discrepancies between the population and individual parameters, and between observations and model predictions, respectively. Both form random variables normally distributed with means equal to 0 and variance equal to ω^2 and σ^2, respectively. As caffeine is not considered a highly variable drug, estimated values of 20% coefficient of variation were used for inter-individual and residual variability. Co-variances between random effects were not considered.

2.6. Computer simulations

The proposed semi-physiological model (Fig. 1), the estimates of the population pharmacokinetic and physiologic parameters (Table 1) and random effect parameters (Table 2) were codified in control files that were launched using NONMEM VI software to simulate the plasma concentration *vs.* time profiles after caffeine administration. Each simulation was performed in 24 individuals in the studies conducted in healthy subjects. In the case of cirrhotic patients, 12 individuals were included in the simulations, following the original work (Marco Carbayo, 2008). The plasma concentration–time data obtained from simulations were imported to Excel to draw the concentration–time curves for the comparison to the published experimental data.

2.7. Model validation

Each plasma concentration–time curve obtained by simulation was drawn together with the experimental plasma concentration–time data taken from each original paper (Haller et al., 2002; Marco Carbayo, 2008; McLean and Graham, 2002; Turpault et al., 2009). Original data from Turpault et al. (2009) and Haller et al. (2002) were obtained by scanning the experimental curves with Portable GetData 2.24 software. Original data from Marco Carbayo (2008) and McLean and Graham (2002) were directly obtain from tables. The curves were compared visually in order to validate the proposed model, which aimed to show the behavior of caffeine in humans.

3. Results

Figs. 2–5 present plasma concentration–time curves of caffeine and paraxanthine at the different dose levels studied. Individual results of simulations were obtained using parameters from

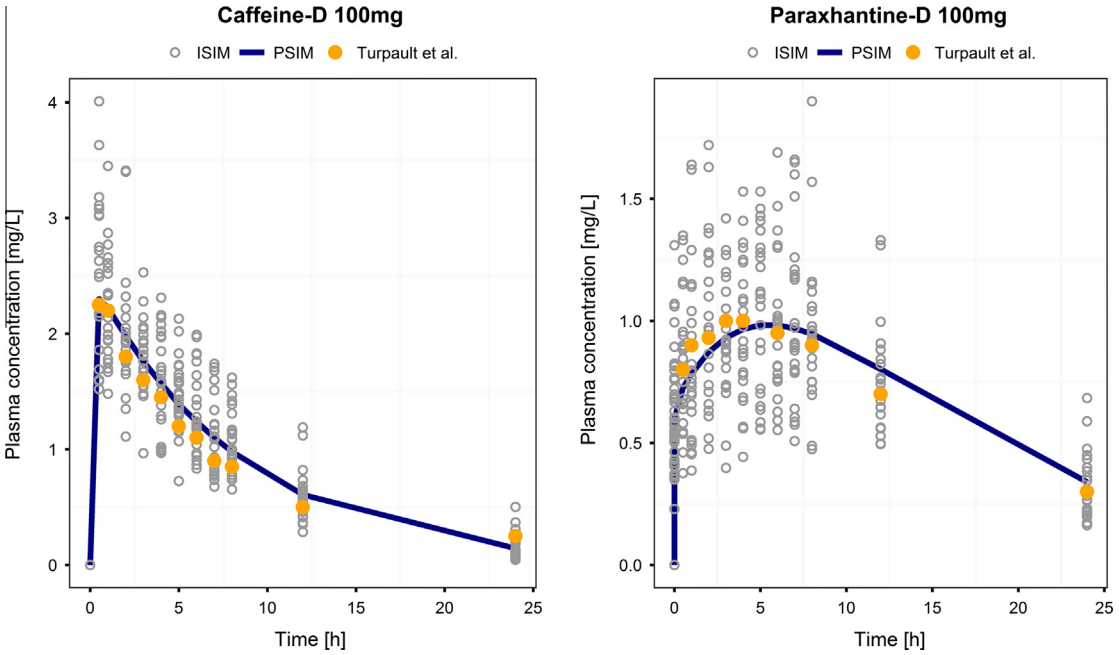

Fig. 2. Population and individual simulated plasma concentrations (mg/L) vs. time (h) of caffeine (left) and paraxanthine (right) when a dose of 100 mg of caffeine base is administered. Grey dots represent the individual simulation data, blue line the population simulation profile and orange dots the data from Turpault et al. (14). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

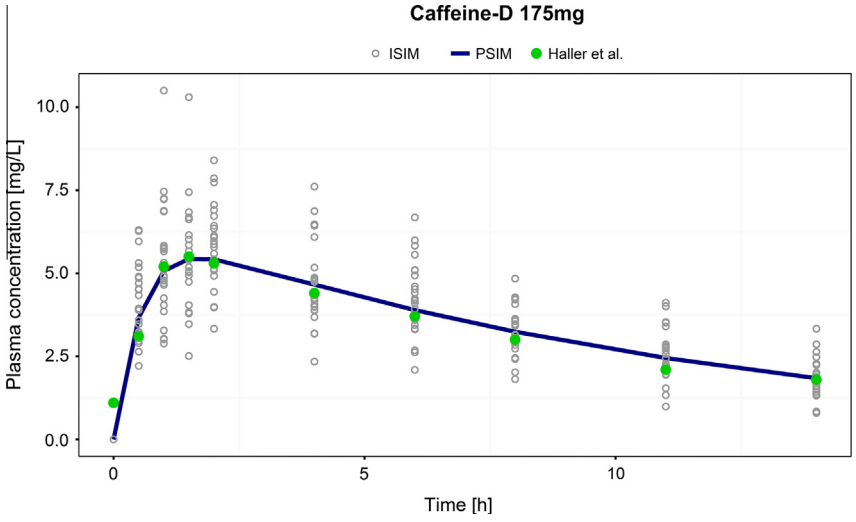

Fig. 3. Population and individual simulated plasma concentrations (mg/L) vs. time (h) of caffeine when a dose of 175 mg of caffeine base is administered. Grey dots represent the individual simulation data, blue line the population simulation profile and green dots the data from Haller et al. (15). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1 with variability (Table 2) while population results of simulation were obtained using parameters of Table 1 without variability (namely the average data).

Fig. 2 shows plasma concentration–time curves of caffeine and paraxanthine, respectively when a dose of 100 mg of caffeine base is administered as an oral solution and population parameters from (Turpault et al. (2009) are used, in comparison to plasma concentration–time curves from Turpault et al. (2009).

Fig. 3 shows plasma concentration–time curves of caffeine when a dose of 175 mg of caffeine base is administered as an oral solution and population parameters from Haller et al. (2002) are used, in comparison to plasma concentration–time curves from Haller et al. (2002).

Fig. 4 shows plasma concentration–time curves of caffeine and paraxanthine respectively when a dose of 400 mg of caffeine base is administered as an oral solution and population parameters from McLean and Graham (2002) are used, in comparison to plasma concentration–time curves from McLean and Graham (2002).

Fig. 5 shows plasma concentration–time curves of caffeine when a first dose of 100.6 mg of caffeine base is administered intravenously in bolus and a second dose of 200 mg or 250 mg (depending on the subject weight) of caffeine base is administered as an oral solution, using population parameters from Marco Carbayo (2008), in comparison to plasma concentration–time curves from Marco Carbayo (2008).

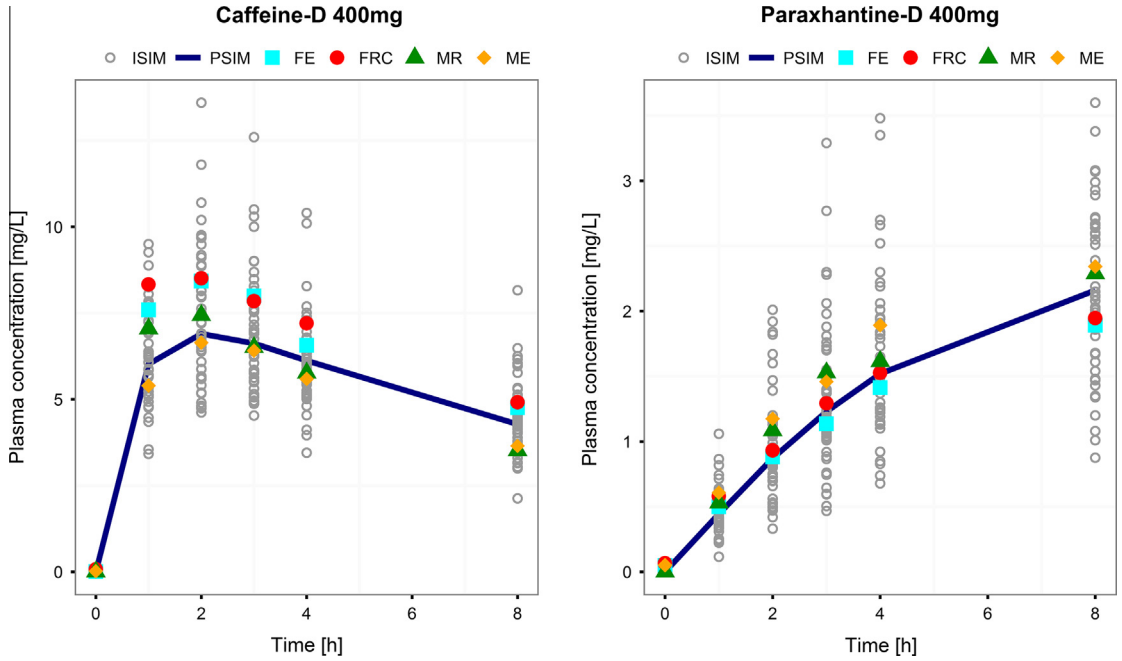

Fig. 4. Population and individual simulated plasma concentrations (mg/L) vs. time (h) of caffeine (left) and paraxanthine (right) when a dose of 400 mg of caffeine base is administered. Grey dots represent the individual simulation data, blue line the population simulation profile and data from McLean and Graham are represented (7). FE: women in exercise trial; FRC: women in resting trials; MR: men in resting trial; ME: men in exercise trial.

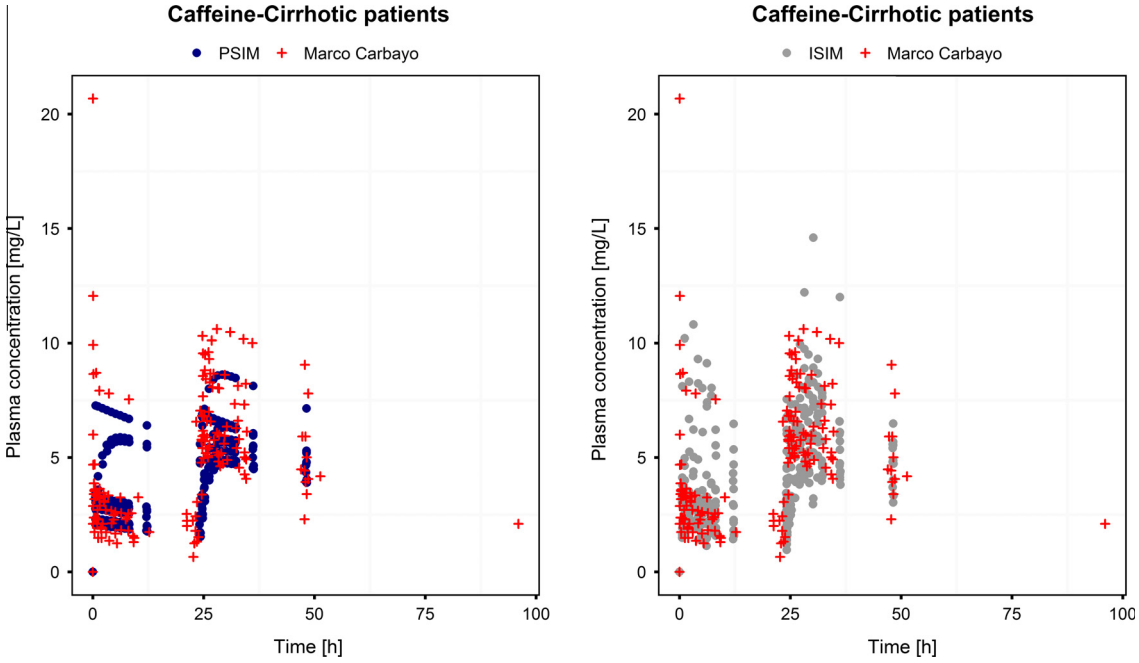

Fig. 5. Population (left) and Individual (right) simulated plasma concentrations (mg/L) (grey dots) vs. time (h) of caffeine when a first dose of 100.6 mg of caffeine base is administered intravenously in bolus and a second dose of 200 mg or 250 mg (depending on the subject weight) of caffeine base is administered as an oral solution, using population parameters from Marco Carbayo (16) (red dots). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

The proposed semi-physiological model was studied to represent the pharmacokinetic behavior of caffeine in humans, both in healthy subjects and patients suffering from cirrhosis. This semi-physiological model was previously used to perform a simulation exercise of bioequivalence studies in order to select the best analyte (parent compound or metabolite) to assess the pharmaceutical performance of drug formulations (Fernandez-Teruel et al., 2009a,b; Navarro-Fontestad et al., 2010). Once it was

demonstrated that in most scenarios parent compound was the best analyte for the mentioned assessment, it was considered essential to validate the model against experimental data.

The validation of the semi-physiological model was performed by challenging simulation results obtained with the model against experimental data from caffeine papers. Caffeine was used as an example for active substance with only hepatic metabolism and with one main metabolite, because it is a compound whose metabolism is well known and of which there were enough experimental data available for the comparison.

Caffeine data found in bibliography for healthy subjects consist of average plasma concentrations *vs.* time profiles, individual plasma profiles were not available. Therefore experimental plots shown in Figs. 2–4 are average data. Fig. 5 shows four experimental data sets, because the original paper (McLean and Graham, 2002) studied four different groups that were compared (men and women after exercise and resting conditions). The lack of individual data in the reference papers did not allow the parameter estimation, but they could be obtained from bibliography, as explained above.

Both caffeine and paraxanthine data were used for the validation to assess the total pharmacokinetic process and the model performance. Experimental curves of paraxanthine were not available neither at dose 175 mg (Haller et al., 2002) nor in cirrhotic patients (Marco Carbayo, 2008). Therefore, the paraxanthine data comparison could not be performed under such conditions.

As it can be observed from Figs. 2 to 5, the simulated individual values and the population prediction overlap with the experimental data in both parent drug and metabolite at the different doses. The population concentration–time data are practically the same in simulated and experimental plots. The individual simulated data showed no trend of disagreement with the experimental values in any part of the plots. The model describes the experimental data of caffeine in the whole range of doses that have been studied, both in healthy and cirrhotic subjects. Therefore, the model is considered to be adequate for drugs without a significant first pass effect and with a single main metabolite formed in the liver.

5. Conclusion

The proposed semi-physiological model has been successfully validated with caffeine as a model compound because it has shown its ability to reproduce adequately the pharmacokinetic behavior of caffeine in both healthy subjects and cirrhotic patients. Therefore the model could be used to simulate plasma concentration–time profiles of drugs with the same pharmacokinetic scheme as caffeine, as long as their population parameters are known, and it could be useful *e.g.* for bioequivalence trial simulation of drugs that undergo hepatic metabolism with a single main metabolite and negligible first pass effect.

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