Use of Physiologically Based Pharmacokinetic Models Coupled with Pharmacodynamic Models to Assess the Clinical Relevance of Current Bioequivalence Criteria for Generic Drug Products Containing Ibuprofen

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ABSTRACT: Physiologically based pharmacokinetic models coupled with pharmacodynamic (PBPK/PD) models can be useful to identify whether current bioequivalence criteria is overly conservative or venturesome for different drugs. A PBPK model constructed with Simcyp Simulator® using reported biopharmaceutics parameters for ibuprofen was coupled with two published PD models: one for antipyresis and one for dental pain relief. Using products with doses of 400 mg and 10 mg/kg as “reference (R)” drug products, virtual products with doses of 280 mg and 7 mg/kg, respectively, could be interpreted as representing bioinequivalent test (T) drug products, as the point estimate for the ratios T/R are well below the bioequivalence limits. Despite being bioequivalent in terms of PK, these lower doses were shown to be therapeutically equivalent to the higher doses because of the flat dose–response relationship of ibuprofen. Sensitivity analysis of the PBPK/PD models demonstrated that gastric emptying time, dissolution rate and small intestine pH are variables that influence ibuprofen PK, but do not seem to significantly affect its PD. It was concluded that current bioequivalent guidance might be unnecessarily restrictive for ibuprofen products. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: Pharmacokinetic/pharmacodynamic models; ibuprofen; dissolution; Biopharmaceutics; bioequivalence; biowaiver; dose–response

INTRODUCTION

In 1995, the US Food and Drug Administration (FDA) incorporated the fundamentals of the Biopharmaceutics Classification System (BCS) into its legal framework.1 Since then, many other regulatory authorities and the World Health Organization (WHO) have published their own guidelines related to the BCS-based biowaiver.2–5 However, the extension of BCS-based biowaiver decisions to drugs belonging to other BCS classes, other than those showing high solubility and high permeability, has not yet reached a consensus among regulators. The most controversial issue is about biowaiving drug products containing weakly acidic drugs that exhibit high solubility at pH 6.8 and are highly permeable, as suggested by WHO.3 Although it is expected that such drugs would behave like Class 1 drugs in the proximal intestine, as the dose would completely dissolve under intestinal conditions, Class 2 weak acids showed a higher risk for bioinequivalence for Cmax.6,7

Ibuprofen, a widely used nonsteroidal anti-inflammatory drug, is a classic representative of Class 2 weakly acid drugs as it is almost completely absorbed and it has a dose number lower than 1 at pH 6.8 at 37 °C.8,9 A consensus as to whether a BCS-based biowaiver decision is advisable has not yet been reached.9–13 Some authors have reported that the in vitro set of dissolution testing, as required by all BCS-based biowaiver guidelines, was not able to predict the in vivo bioequivalence outcome for drug products containing ibuprofen, showing false-positive results (similar dissolution profiles for nonbioequivalent drug products)11 and a false-negative result (nonsimilar dissolution profile for a bioequivalent drug product).12 In this context, false-positive results represent the consumer risk, which is the main concern of regulatory authorities, and should be carefully addressed. The two nonbioequivalent results reported were because of Cmax differences between T and R formulations and not to differences in the extent of exposure,11 corroborating the conclusions taken in the meeting report of the Workshop “Bioequivalence, Biopharmaceutics Classification System, and Beyond.”6 In 2007, it had already been pointed out that to grant a biowaiver for some Class 2 weakly acid drugs, it would be necessary to widen the bioequivalence limits for Cmax, if it is not critical to the therapeutic efficacy of the drug, in line with the WHO guidance.3,6 Given that the bioequivalence criteria were empirically defined, based on the opinions of FDA medical experts that only differences of greater than 20% for Cmax and AUC0–t would be significant for all drug products,14 it would not be surprising to find overly conservative scenarios. Owing to the sigmoidicity factor of the dose–response curve, formulation differences in absorption could be either attenuated or magnified in terms of the PD response.15 When the concentrations resulting from the recommended dosage range are higher than the concentration at which the effect is half-maximal (EC50), formulation differences are expected to be attenuated in terms of PD response.

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Physiologically based pharmacokinetic (PBPK) models coupled with or without in vitro-in vivo extrapolation techniques have already been used to justify extending BCS-based biowaiver decisions to some Class 2 drugs. However, to the best of our knowledge, pharmacokinetic/pharmacodynamic (PK/PD) models have not been applied for this purpose to date.

The goal of the present analysis was to fit PBPK/PD models for ibuprofen into Simcyp Simulator, using published biopharmaceutics, PK and PD data, to evaluate whether the bioequivalence criteria would indeed be clinically significant for such a drug. The clinical indications considered were fever reduction in children and dental pain relief in adults, for which PD models have already been established. In this context, the risks of giving in vivo bioequivalence studies for immediate-release oral dosage forms containing ibuprofen were also assessed.

**EXPERIMENTAL**

**Computer Hardware and Software**

Simcyp Simulator® version 12.2 (Simcyp Ltd., Sheffield, UK) was run using a DELL computer with Intel Core™ i5 processor (DELL, Hortolândia/SP, Brazil).

**Virtual Populations**

An effect of age on the PK of ibuprofen has been proposed by some authors, but this could not be substantiated by other authors. However, it has been reported that the PD response elicited by ibuprofen is age dependent. For these reasons, the age range of the virtual population was selected to closely match those of the subjects enrolled in two clinical trials: (1) children aged from 2 to 11 years for the antipyretic model and (2) adults aging 18–40 years for the dental pain relief model.

**Data Used for Simulations of PD Response of Ibuprofen**

All physicochemical properties, biopharmaceutics, PK and PD parameters of ibuprofen, unless otherwise stated, were taken from the literature and are summarized in Table 1.

**PBPK Model**

**Absorption**

The oral absorption of ibuprofen was predicted using the advanced dissolution, absorption, and metabolism (ADAM) model, which divides the gastrointestinal tract (GI) into nine segments. Negligible absorption from the stomach was assumed and the default settings of the software for gastric emptying time (GET; based on first-order emptying with a half-life of 16.6 min), small intestine transit time (based on a Weibull probability distribution function), and GI pH (default pH values: duodenum = 6.4; jejunum I = 6.5; jejunum II = 6.6; ileum I = 6.8; ileum II = 7; ileum III = 7.1; ileum IV = 7.3; and colon = 6.5) were used to establish the absorption model.

The effective permeability in humans (P_{eff,max}) of ibuprofen was estimated using the mechanistic P_{eff} model in ADAM and data obtained from an in vitro permeability study in Caco-2 cells, in which 36 different compounds using the same experimental protocol were investigated, including internal standards of high (e.g., propranolol) and low (e.g., cimetidine) permeability. Because ibuprofen permeability values throughout the small intestine are not statistically different

### Table 1. Parameter Values Used for Ibuprofen Simulations in the Simcyp Simulator

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Reference/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight (g/mol)</td>
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<td></td>
</tr>
<tr>
<td>log P</td>
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<tr>
<td>Compound type</td>
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<td>Solubility at pH 1.2 (mg/mL)</td>
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<tr>
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<tr>
<td>Model</td>
<td>ADAM</td>
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<tr>
<td>P_{eff,max}(10^{-4} cm/s)</td>
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<td>Predicted using the MechP_{eff} model</td>
</tr>
<tr>
<td>P_{app,Caco-2} (10^{-6} cm/s)</td>
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<td>Based on MechP_{eff} prediction</td>
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<tr>
<td>Fraction absorbed</td>
<td>0.99</td>
<td>Based on MechP_{eff} prediction</td>
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<tr>
<td>k_{a} (h^{-1})</td>
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<tr>
<td>Model</td>
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<td>Predicted using the Rodgers and Rowland method</td>
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<td>Fraction unbound plasma</td>
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<td>k_{in} (h^{-1})</td>
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<tr>
<td>k (h^{-1})</td>
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</tr>
<tr>
<td>E_{max}</td>
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<tr>
<td>E_{C50} (mg/L)</td>
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</tr>
<tr>
<td>k_{inh} (h^{-1})</td>
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<td>21</td>
</tr>
<tr>
<td>Sigmoidicity (n)</td>
<td>2.00</td>
<td>21</td>
</tr>
</tbody>
</table>
and considering that the large bowel plays an important role in the extent of ibuprofen absorption, the same $P_{\text{eff,man}}$ value was assumed throughout the seven small intestine segments (from duodenum to ileum IV) and in the colon.

**Distribution**

Upon reaching the portal vein, the PK of ibuprofen was predicted with a full PBPK model. The volume of distribution at steady state ($V_{ss}$) as well as the tissue-plasma partition coefficients ($k_p$) were predicted using the Rogers and Rowland method. The predicted $V_{ss}$ was further optimized to 0.12 L/kg by applying a global $k_p$ scalar of 0.55 (children) and 0.45 (adults), to best fit the $C_{\text{max}}$. In spite of the empirical adjustment, the predicted $V_{ss}$ was still within the observed range of the apparent volume of distribution of ibuprofen, 0.1–0.2 L/kg. Additionally, the unbound fraction of 1% reported by Aarons et al. was applied.

**Elimination**

Ibuprofen is administered as a racemic mixture and 60% of the R-ibuprofen undergoes in vivo unidirectional chiral inversion to the S-ibuprofen; thus, 80% of the administered dose is bioavailable as S-ibuprofen. The oxidative metabolism, 2 and 3-hydroxylation, and direct glucuronidation of the S-ibuprofen accounts for around 90% of its clearance. CYP2C9 is the main enzyme involved in 3-hydroxylation of ibuprofen, however, CYP3A4, CYP2C8, CYP2C19, and CYP2E1 also play a role in its 2-hydroxylation. As the S-ibuprofen represents the major bioavailable fraction of the drug after administering it as a racemate, the in vitro intrinsic clearance (CLint) values for CYP2C9, CYP2C8, CYP3A4, CYP2C19, and CYP2E1 were back-calculated using the product of the percentage of total normalized rate for each P450 form [enzyme turnover/reported specific content of each P450 form in human liver microsomes (HLMs)] and the sum of CLint1-2 for 2 and 3-hydroxylation of S-ibuprofen incubated with HLMs. These were entered into the simulator with the aim of mechanistically predicting ibuprofen oral clearance using software-default intersystem extrapolation factors. Moreover, the kinetics of ibuprofen glucuronidation by UGT1A3, UGT1A9, UGT2B4, and UGT2B7 were added to the PBPK model. As the calculated weighted arithmetic mean of ibuprofen oral clearance (Cl/F) for children is higher than that for adults, 0.086 and 0.054 L/h/kg (calculated using the review by Davies), we added an additional Clint of 22 μL/min.mg microsomal protein to achieve the best fit for the pediatric PK profile. The resultant predicted Cl/F values, 0.082 (children) and 0.060 L/h/kg (adults), were practically identical to the observed ones.

**PD Models**

**Antipyretic Model**

Pharmacodynamic parameters inputted to Simcyp Simulator were collected from a published PK/PD model that was based on a Phase 3 study conducted in 103 febrile children. As there was a temporal disconnection between the mean time of maximum drug concentration in plasma ($T_{\text{max}}$, ranging from 0.5 to 1.9) and the time to reach the maximum pharmacological response (around 3 h), graphically represented by an anticlockwise hysteresis in the PK/PD profile, the authors found that the antipyretic effect of ibuprofen would be better described by an indirect PD response model, represented by the following equation:

$$\frac{dT}{dt} = k_{in} \left[1 - \left(\frac{C_{\text{max}}}{C_t + EC_{50}}\right)\right] - k_{out} T$$

where $dT/dt$ is the rate of change in body temperature, $T$ is body temperature, $C_t$ is drug concentration at time $t$ after ibuprofen administration, $E_{\text{max}}$ represents the maximum effect of ibuprofen, $k_{in}$ and $k_{out}$ are, respectively, the zero- and first-order rate constants of formation and degradation of prostaglandin-E2, and $n$ is the sigmoidicity factor. To achieve a better fit to the observed data, a lower $E_{\text{max}}$ value was entered into the simulator because slightly hypothermic conditions (data not shown) would occur if the reported value of 0.055 was used.

**Dental Pain Relief Model**

Pharmacodynamic parameters were taken from the reported PK/PD model that had been derived from a placebo-controlled clinical trial that enrolled 282 adults submitted to surgical extraction of at least 2 third molars. In this report, the delay between plasma and effect-site concentration was addressed by using an effect compartment model, and the PD response was described as the sum of placebo and drug effects:

$$\text{PR}(t) = f_p(t) + f_d(C_e)$$

and

$$f_p(t) = P_{\text{max}} \left(1 - e^{-kt}\right)$$

$$f_d(C_e) = \frac{E_{\text{max}}C_e}{C_e + EC_{50}}$$

$$\frac{d(C_e[t])}{dt} = k_{0}(C_p[t] - C_e[t])$$

where PR(t) is the pain relief score at a given time $t$, $P_{\text{max}}$ is the maximum placebo effect, $k$ is a first-order rate constant for the placebo effect, $C_e$ is the concentration of ibuprofen at effect-site, $C_p$ is the ibuprofen plasma concentration, $k_{0}$ is the first-order plasma effect-site equilibrium rate constant, and $n$ is the sigmoidicity factor.

**Simulations**

Simcyp Simulator version 12.2 (Simcyp Ltd.) was used to predict the PK and PD profiles for ibuprofen. Virtual populations were selected to closely match the populations enrolled by Walshon et al. or by Li et al. with regard to age range and gender ratio. The virtual trials were performed with 8 h monitoring for ibuprofen administered as suspension or tablets. The following simulations were performed:

1. For the antipyretic response, two virtual trials were conducted enrolling 100 children aging 2–11 years per trial: dosing (a) 5 or (b) 10 mg/kg of ibuprofen suspension. To approximate the behavior of the administered suspension, the appropriate dose of ibuprofen was entered into the simulator as a solution, assuming that because of its low solubility at acidic pH typical of the fasted stomach, it would immediately precipitate to a suspension with a mean particle radius of 25 μm and a log-normal particle size distribution.
size distribution. The simulated PK and PD profiles were additionally compared with a different set of published data\textsuperscript{24} to evaluate the external validity of the model.

2. For the dental pain relief response, a virtual trial was conducted enrolling 100 adults aging 18–40 years and receiving a single oral dose of 400 mg of ibuprofen tablets. The predicted data were compared with the observed ones\textsuperscript{21} to internally validate the model, as no other report describing such PK and PD profiles for the same sampled population was found.

3. Additional virtual trials were conducted for both PD responses to establish dose–response relationships as follows:

   (a) For the antipyretic model, virtual trials were conducted with 100 children per trial aging 2–11 years receiving (a) 2, (b) 5, (c) 7, or (d) 10 mg/kg of ibuprofen suspension.
   (b) For the dental pain relief model, virtual trials were conducted with 100 adults per trial aging 18–40 years receiving (a) 100, (b) 200, (c) 280, or (d) 400 mg of ibuprofen tablets.

In this set of simulations, the doses of 10 mg/kg and 400 mg were taken to be the “reference,” whereas the 7 mg/kg and 280 mg doses, respectively, were chosen deliberately to fall outside the limits of bioequivalence (i.e., 80%–125%, for log-transformed data).

4. Sensitivity analysis. The effect of four different input variables over the PK and PD responses of ibuprofen was evaluated using the one-at-a-time variation approach. Gastric solubility (ranging from 0.038 to 3.2 mg/mL), GET (ranging from 0.1 to 0.8 h), apparent permeability coefficient ($P_{app}$) obtained from Caco-2 monolayers (ranging from $5 \times 10^{-5}$ to $10^{-4}$ cm/s), and small intestine pH (ranging from default values to “default minus 2” pH units throughout duodenum-ileum IV segments of ADAM) were tested.

5. The effect of different dissolution rates (ranging from 85% dissolved in 5 min to 85% dissolved in 90 min) over the PK and PD responses of ibuprofen was evaluated in five virtual trials enrolling 100 adults aging 18–40 years receiving 400 mg of ibuprofen tablets (dental pain relief model).

Statistical Analysis

Groups were compared using analysis of variance ANOVA one-way with Bonferroni correction to adjust for multiple comparisons. A $p$ value of less than 0.05 was considered significant.

RESULTS

The first set of virtual trials was used to establish and provide external validation, where possible, for the models. Figures 1 and 2 show the mean predicted and observed PK and PD profiles for antipyretic and dental pain relief models. All correlation coefficients were statistically significant ($p < 0.01$), with $R^2$ values of 0.8 or higher. These results confirm that the PBPK and PD model setups adequately describe the PK and PD data observed in the pediatric and adult studies from the literature.

Figure 1. (a) Mean observed (open squares; mean ± SE)\textsuperscript{24} and model-predicted PK profile (solid line) for children receiving 5 mg/kg of ibuprofen suspension. (b) Mean observed (open squares; mean ± SE)\textsuperscript{24} and model-predicted PK profile (solid line) for children receiving 10 mg/kg of ibuprofen suspension. (c) Mean observed (open squares; mean ± SE obtained by digitizing the original figures)\textsuperscript{21} and model-predicted PK profile (solid line) for adults receiving 400 mg ibuprofen tablets. Dotted lines represent 95% CI (upper and lower limits) for predicted PK profiles. Pearson correlation coefficients ($r^2$) were calculated comparing mean observed versus predicted values.
As the models performed adequately in the simulator, further simulations were made, aiming to assess how sensitive the antipyretic and dental pain relief responses are to changes in the ibuprofen peak exposure as well as extent of exposure.

The second series of virtual trials was conducted to examine the dose proportionality of the PK and PD responses to ibuprofen. Ibuprofen PK was dose-proportional over the tested dose ranges, 100–400 mg (Figs. 3a and 3c). These results correspond to the literature reports of linear PK for ibuprofen over the dose range of 200–400 mg.⁸ Although nonlinearity at higher doses has been reported for total ibuprofen plasma concentrations, this is more likely due to saturation of plasma protein binding than reduced absorption, given that urinary recovery was dose independent.⁸,⁴²

A positive dose–response relationship was observed for dental pain relief, as when evaluated by maximum pain relief score (Rₘₐₓ) as well as extent of pain relief (AUCR), oral ibuprofen doses of 100, 200, and 400 mg were significantly different from each other (Fig. 3d). Likewise, oral administration of 2, 5, and 10 mg/kg resulted in significant differences among extent of antipyretic response, represented by the area under the R(0)-subtracted response curve over 0–8 h (AUCRₘᵢₓ) (Fig. 3b). Although the 5 and 10 mg/kg doses were significantly superior to 2 mg/kg in terms of maximum decrease from the initial body temperature (Rₘᵢₙ), they were not different from each other (Fig. 3b). Nor were doses of 7 or 10 mg/kg of ibuprofen suspension to febrile children significantly different from each other. Likewise, the comparisons involving the simulated administrations of 280 versus 400 mg of ibuprofen tablets to adults, in whom third molars were extracted, did not show any significant PD differences. Figure 4 shows the predicted concentration–response curves obtained when simulating the administration of 10 mg/kg of ibuprofen suspension (Fig. 4a) and 400 mg of ibuprofen tablets (Fig. 4b). Visual analysis of the maximum response regions of the plots reveals that both PD responses are practically flat and, thus, insensitive to the PK changes: from 35 to 15 mg/L, in case of antipyretic response (whose EC₅₀ is around 6.18 mg/L) and from 12 to 23 mg/L, in case of dental pain relief response (whose EC₅₀ is around 10.2 mg/L). Interestingly, such PK ranges contain the Cₘᵢₓ values obtained after simulating administration of the lowest therapeutic doses (5 mg/kg and 200 mg), again indicating a flat concentration–response relationship for ibuprofen.

In the third set of virtual trials, sensitivity analysis of the models was conducted to determine which parameters might be important to the PK and/or PD response to ibuprofen. Sensitivity analysis demonstrated that the variables gastric solubility and Pₘₐₓ over the range tested, did not significantly affect the PK or PD profiles (data not shown). By contrast, accelerating GET to 0.1 h shortened the PK Tₘᵢₓ for adults (from 1.40 to 1.04 h) and children (from 1.22 to 0.83 h), whereby Cₘᵢₓ increased by 8% and 13% in adults and children, respectively, but with little or no effect on AUC₀–₈. The opposite effect, an increase in Tₘᵢₓ and a decrease in Cₘᵢₓ was observed when GET was delayed to 0.8 h (Table 2). Assuming a very acidic pH in the small intestine also significantly affected the absorption rate of ibuprofen. Reducing the pH over the entire small intestine also significantly affected the absorption rate of ibuprofen. Reducing the pH over the entire small intestine by 2 pH units from the ADAM default pH profile (a quite extreme condition, physiologically speaking) delayed Tₘᵢₓ and decreased Cₘᵢₓ around 25%, whereas AUC₀–₈

**Figure 2.** (a) Mean observed (open squares; mean ± SE)⁴⁴ and model-predicted PD profile (solid line) for children receiving 5 mg/kg of ibuprofen suspension. (b) Mean observed (open squares; mean ± SE)⁴⁴ and model-predicted PD profile (solid line) for children receiving 10 mg/kg of ibuprofen suspension. (c) Mean observed (open squares; mean ± SE obtained by digitizing the original figures)⁴¹ and model-predicted PD profile (solid line) for adults receiving 400 mg ibuprofen tablets. Dotted lines represent 95% CI (upper and lower limits) for predicted PD profiles. Pearson correlation coefficients (r²) were calculated comparing mean observed versus predicted values.
was only slightly affected, by less than 7% (Table 2). Restricting the two unit decrease in pH to the duodenum (a somewhat more realistic variation in intestinal pH) resulted in no effect on either peak or extent of ibuprofen exposure. Even though the absorbed fraction from the “acidified” duodenum was reduced from 22% to 11%, the absorption from the jejunum increased from 69% to 78%, this compensating the proximal acidification completely.

Nevertheless, although GET and small intestine pH affected the absorption rate of ibuprofen, the peak and extent of antipyretic and dental pain relief responses were not changed. Only the time to achieve maximal PD responses was affected by these variables, increasing when the small intestine pH was acidified and when gastric emptying was slower (Table 3).

Different dissolution rates did not affect the magnitude of C_{max}, nor AUC_{0-8} (Fig. 5a). T_{max} values were the same when Q > 85% in 5, 15, and 30 min. When very slowly dissolving tablets, Q > 85% in 90 min, were simulated to represent an extreme case, the T_{max} was delayed to 2.2 h, which translated into a delay in the onset of dental pain relief by 30 min when compared with the response elicited by simulating the case of extremely rapidly dissolving tablets, Q > 85% in 5 min (Fig. 5b). This result can be attributed to a change in the regional distribution of dose absorption over the eight ADAM intestinal segments, even though the fraction of drug absorbed is the same, around 99% (Fig. 5c).

**DISCUSSION**

**Bioequivalence Criteria Versus Therapeutic Equivalence: Antipyresis**

As the bioequivalence criteria were empirically defined, it seems reasonable to expect that they might be overly conservative for some drugs and venturesome, even risky, for other drugs. In fact, for these reasons, some regulatory agencies allow widened C_{max} limits in cases of highly variable drugs, provided that concerns about safety and efficacy are not anticipated, but recommend that the acceptance interval for AUC and C_{max} be tightened for narrow therapeutic index drugs.

Ibuprofen is not a highly variable drug, its within-subject coefficient of variation (CV) is around 16%, so widening bioequivalence acceptance limits for comparisons involving C_{max} does not come under consideration within the current legal framework. However, because of the indirect nature of the antipyretic effect elicited by ibuprofen, it appears that its PD response is not very sensitive to changes in dose and consequently in PK profile.

In the simulations, a dose–response for AUC_{corr} was observed through the ibuprofen doses of 2, 5, and 10 mg/kg when a low-temperature scenario (initial temperature around 39.1 °C) was invoked. However, two published clinical trials showed a different scenario. In the first study, enrolling 118 febrile children aging 2–11 years, mean maximum percent temperature
Figure 4. Predicted concentration–response curves for (a) febrile children receiving 10 mg/kg of ibuprofen suspension and (b) adults submitted to surgical extraction of third molars receiving 400 mg of ibuprofen tablets. Vertical and horizontal lines represent, respectively, $C_{\text{max}}$ and $R_{\text{max}}$ or $R_{\text{min}}$ after simulated intake of 5 mg/kg or 200 mg of ibuprofen (dotted lines) and 7 mg/kg or 280 mg of ibuprofen (dashed lines).

reduction from the baseline value and the area under the percent reduction curve, representing the extent of antipyretic response, were not meaningfully different when comparing doses of 5 and 10 mg/kg in a group of children with “low” fever temperatures (38.3–39.1 °C). In the other study, which enrolled 163 febrile children aging 0.25–11 years, it was observed that although administration of 5 and 10 mg/kg were both superior to placebo in terms of maximum decrease from the initial temperature and AUCR$_{\text{corr}}$, these doses were not different from each other, regardless of the initial temperature. Despite the lack of statistical difference in these parameters between the two doses, the authors concluded that, based on three other AUC endpoints, including the AUC of the change in temperature required to reduce baseline to normal versus time (AUC $\Delta$Temp$_{\text{r}}$), the 10-mg/kg dose was more effective. Nevertheless, when considering the low-temperature group, even AUC $\Delta$Temp$_{\text{r}}$ values were not significantly different between the two tested doses.

Such apparent divergence between predicted and observed dose–response relationship might be because of the greater sample size in each simulated dose group than in the reported clinical trials as well as an underestimation of the variability in the simulations. Even though the observed $k_{\text{out}}$ showed a CV around 78%, no variability was inputted to the Simcyp Simulator as the variability of $T_{0}$ was reported to be low, most likely the values of $k_{\text{in}}$ and $k_{\text{out}}$ are correlated, which cannot be accounted for at present in software, so using this CV value might have overestimated interindividual variability and biased the conclusions. Even though the interindividual variability was underestimated in the simulations (CV for $k_{\text{in}}$ and $k_{\text{out}}$ were set to 0), AUCR$_{\text{corr}}$ and $R_{\text{min}}$ were not statistically different when doses of 7 and 10 mg/kg of ibuprofen suspension were simulated in febrile children (Fig. 3b). Considering 10 mg/kg as the R drug product, 7 mg/kg would have to be interpreted as a bioinequivalent T drug product in terms of PK, as the point estimates for the ratios $C_{\text{max,T}}/C_{\text{max,R}}$ and $AUC_{\text{T}}/AUC_{\text{R}}$ are around 0.7 and consequently far below the bioequivalence limits. Despite being bioinequivalent, the 7-mg/kg dose was simulated to be therapeutically equivalent to the higher dose in terms of the antipyretic response. This conclusion is underlined by the results of a published clinical trial showing that administration of 7 mg/kg of ibuprofen suspension or 400 mg of ibuprofen as effervescent granules (equivalent to a dose of 11.8 mg/kg, taking the mean body weight of enrolled patients into consideration) to 103 febrile children aging 4–16 years resulted in superimposable antipyretic profiles, plotted together after digitizing the original dataset. This outcome was observed regardless of whether the initial temperature was low or high.

Bioequivalence Criteria Versus Therapeutic Equivalence: Dental Pain Relief

The dental pain relief scenario presented similar findings. A dose–response relationship in terms of $R_{\text{max}}$ and AUCR was observed in the simulations over the range of 100–400 mg of ibuprofen (Fig. 3d), which is consistent with the results published by Schou et al. They reported a positive dose–response relationship of ibuprofen over the range 50–400 mg in terms of mean and total pain relief scores (PAR and TOTPAR) and sum of pain intensity difference (SPID) observed in 304 patients whose impacted mandibular third molars were surgically removed. Interestingly, the doses of 200 and 400 mg were not different from each other when evaluating TOTPAR.

A lack of significant differences in the effects of 200 and 400 mg of ibuprofen have also been reported in a study enrolling 161 patients undergoing surgical removal of lower bony impacted third molars. Two other single-dose, placebo-controlled, double-blind, randomized, parallel-group clinical
Hence, suprabioavailability and associated toxicity is achieved in the case of ibuprofen. Sensitivity Analysis for PK Parameters (mean) of ibuprofen suggest that the differences in the dose range 200–600 mg revealed that 14 studies failed to demonstrate bioequivalence because of small \( C_{\text{max}} \) differences (although AUC fulfilled the BE criteria in all cases), suggesting that weakly acid drugs belonging to BCS Class 2 show a higher risk for bioequivalence due to peak, but not extent, of exposure. So, concerns might arise around the effect of decreasing \( C_{\text{max}} \) (sub-bioavailable drug products) on the peak effect of the PD response as well as increasing \( C_{\text{max}} \) (suprabioavailable drug products) on adverse reactions to ibuprofen.

Trials enrolling 120 and 100 patients whose third molars were extracted reported no significant differences between 200 and 400 mg in terms of TOTPAR, onset of analgesic effect and SPID. Significant differences were observed only in the time of first perceptible relief in those patients who experienced meaningful relief, in the percentage of patients rating the study medication as very good or excellent and in the time to remedication. However, the differences observed in the overall evaluation of the treatments in the single-dose study could not be detected in the multi-dose study.

A meta-analysis utilizing data collected from 13 reported studies of immediate-release tablets containing ibuprofen over the dose range 200–600 mg revealed that 14 studies failed to demonstrate bioequivalence because of small \( C_{\text{max}} \) differences (although AUC fulfilled the BE criteria in all cases), suggesting that weakly acid drugs belonging to BCS Class 2 show a higher risk for bioequivalence due to peak, but not extent, of exposure. So, concerns might arise around the effect of decreasing \( C_{\text{max}} \) (sub-bioavailable drug products) on the peak effect of the PD response as well as increasing \( C_{\text{max}} \) (suprabioavailable drug products) on adverse reactions to ibuprofen.

Even though coadministration of food decreases ibuprofen \( C_{\text{max}} \) around 30%–50% and delays \( T_{\text{max}} \) achievement in 30–60 min, the labeling of ibuprofen suspension and tablets indicate it can be taken with or without food, suggesting that such a decrease in the PK response to ibuprofen is not considered clinically relevant. Thus, it seems safe to conclude that the risk of undermedication associated with administration of sub-bioavailable drug products (within a certain range, of course) is low. With regard to suprabioequivalent products, it is noted that although the highest single therapeutic dose indicated on the Prescriber’s Information for ibuprofen drug products is 800 mg (equivalent to ~11.5 mg/kg assuming an average weight of 70 kg in adults) for rheumatoid arthritis, ingestions of up to 99 mg/kg of ibuprofen did not result in serious adverse event. Hence, suprabioavailability and associated toxicity is not a critical factor—it is not a narrow therapeutic index drug.

Furthermore, it seems highly likely that ibuprofen will have a flat dose–response for other prostaglandin-dependent ailments, such as rheumatoid arthritis, as the IC\(_{50}\) value of...
ibuprofen for cyclooxygenase-2 in human interleukin-1β-stimulated synovial cells is more than 10-fold lower than the mean plateau concentration of ibuprofen in synovial fluid from patients with arthritis after taking a single 400 mg oral dose (0.7 vs. 8.2 mg/L).56,57 Indeed, it has already been reported that administrations of 200 or 400 mg q.i.d to 20 patients with rheumatoid arthritis did not translate into differences clinically significant in terms of articular index and pain score.58 Thus, it seems feasible to extrapolate the findings predicted using the PBPK/PD models to other scenarios not specifically addressed in the current work. Additionally, it is important to highlight that $E_{\text{max}}$ as well as $EC_{50}$ estimated from clinical outcome versus time curves (our case) do not represent only one specific PD mechanism (e.g., inhibit cyclooxygenase-1), but all actions elicited by ibuprofen to achieve the clinical response measured, even unknown ones.

Finally, given the low risk of therapeutic failure because of sub- or suprabioavailable drug products, the current bioequivalence criteria for $C_{\text{max}}$ appears to be overly conservative for drug products containing ibuprofen. Even though it is not a highly variable drug, it seems safe to widen its acceptance interval with regard to $C_{\text{max}}$ as moderate differences in this PK parameter do not appear to translate into clinical differences.

**Sensitivity Analysis: Gastric Solubility and GET**

Sensitivity analysis demonstrated that gastric solubility, over the tested range, did not significantly affect PK profile of ibuprofen. Nevertheless, gastric solubility seems to be a relevant variable for ibuprofen absorption, as ibuprofen sodium dihydrate, which can achieve concentrations of around 310 mg/mL when dissolved in a pH 2 solution,59 shows higher $C_{\text{max}}$ and faster $T_{\text{max}}$ than ibuprofen acid,60 the solubility of which is around 0.04 mg/mL at pH 1.2.9 This could be due to gastric absorption of dissolved ibuprofen, as has been demonstrated in rats with a ligated pylorus,61 or because of faster gastric emptying of the solution,62 as initial emptying of water from the stomach is faster than that of radiolabeled gelatin or HPMC capsules of ibuprofen,63 which in turn leads to faster absorption.64 Further, it is possible that the gastric pH is temporarily elevated because of the salt dissolution as well as to the coadministration of water,65 and thus accelerates the gastric emptying.66 As Simcyp Simulator® in the current version does not account for gastric absorption and assumes GET is the same for solutions, solutions with precipitation and immediate-release solid dosage forms (assuming immediate disintegration of the dosage form into fine particles), it was to be expected that no sensitivity to gastric solubility would be detected in the sensitivity analysis. By contrast, after manually modifying GET in the software over the range of 0.1–0.8 h, the predicted $C_{\text{max}}$ could be changed by ±15%. This did not however lead to any change in the PD response (Tables 2 and 3).

**Sensitivity Analysis: Dissolution Rate**

In the simulated scenarios, only very prolonged dissolution times (Q > 85% in 90 min) resulted in a change in the $T_{\text{max}}$, and even under these circumstances, neither the $C_{\text{max}}$, nor AUC of ibuprofen were affected. Other cases where dissolution rate affects the absorption rate, but not the extent of exposure, of highly permeable compounds have already been reported in the literature. For example, Kaus et al.67 showed that the $C_{\text{max}}$ ratio of compounds showing $k_a = 3.0 \text{ h}^{-1}$ (i.e., similar absorption rate to that of metoprolol) fell outside the lower limit of bioequivalence acceptance criteria when the dissolution was slow (Q > 85% in 60 min) and the gastric emptying half-life was faster (0.1 h).67 However, the authors assumed a short small intestine
transit time of 3.33 h combined with no absorption of the drug from the colon. In fact, as ibuprofen is absorbed throughout the entire GI, and mean residence time in the small intestine has been reported to be around 4.5 h,\textsuperscript{31,68} it is expected that the Kaus model would overestimate the potential for bioinequivalence for drugs such as ibuprofen.

Our simulations revealed that unless dissolution rate ($k_d$) is slower than the gastric emptying rate ($k_{ge}$), the predicted ibuprofen absorption will remain unaffected by changing dissolution input. Wagner\textsuperscript{69} had already observed this relationship for weakly acid drugs, coining the term “safe zone” for this range of dissolution behavior. Assuming gastric emptying and dissolution kinetics are first-order processes, simulated $k_d$ ranged from 8.3 to 1.4 h\textsuperscript{−1} (in case of $Q > 85\%$ in 5 and 90 min, respectively) and $k_{ge}$ was set at 2.5 h\textsuperscript{−1}, based on the Simcyp Simulator\textsuperscript{⃝} default value for mean gastric residence time of 0.4 h (which is equivalent to a gastric emptying half-life of 16.6 min) for liquids and small disintegrated particles in the fasted state.\textsuperscript{70,71} In the case of the slowest dissolving drug product in the simulations ($k_d = 1.4$ h\textsuperscript{−1}), only $T_{max}$ was affected, yielding a delay in the onset of dental pain relief from 2.5 to 3 h, but with no change in the $R_{max}$ nor AUCR. Hence, the current rapid dissolving criterion ($Q > 85\%$ in 30 min or $k_d = 4.2$ h\textsuperscript{−1}) at pH 6.8 seems to be adequate to ensure that pharmaceutical issues are not important to \textit{in vivo} performance, but rather that ibuprofen absorption will be limited by gastric emptying.

It has been reported that the \textit{in vitro} set of dissolution testing, as required by all BCS-based bioequivalence guidelines, was not able to predict the \textit{in vivo} bioequivalence outcome for drug products containing ibuprofen, showing two false-positive results.\textsuperscript{11} However, in those studies, the R formulation, but not the T drug products, contained sodium lauryl sulfate,\textsuperscript{72} which would automatically eliminate the T drug products from consideration for the BCS-based bioequivalence, as excipients that might affect bioavailability should be qualitatively\textsuperscript{4,5} and quantitatively the same in T and R drug products, even in case of Class 1 drugs.\textsuperscript{4} As ibuprofen might be absorbed by paracellular as well as the transcellular route, given its low molecular weight and high permeability across rat intestinal membranes in spite of being completely ionized under experimental conditions,\textsuperscript{73} tight junction modulation by anionic surfactants could enhance its absorption.\textsuperscript{74} In accordance with this line of reasoning, the T drug products evaluated by Alvarez et al.,\textsuperscript{11} showed significantly lower $C_{max}$ (90\% confidence intervals did not contain the unit) than R drug product containing surfactant. Hence, concerning BCS-based bioequivalence decisions, a similarity between T and R formulations in terms of such critical excipients would appear to be a suitable regulatory strategy to prevent effects that cannot be addressed by dissolution testing.

**Sensitivity Analysis: Small Intestine pH**

$\text{HCO}_3^−$ and secretin levels increased immediately after instilling 0.1 N HCl into duodenum and jejunum of healthy volunteers\textsuperscript{75,76} and a dose–response relationship of these effects has been demonstrated in dogs.\textsuperscript{77} Infusions of up to 42 mmol of 0.1 N HCl in duodenum bulb were completely neutralized before reaching upper jejunum (the aspiration tip was positioned at the ligament of Treitz) of fasting healthy volunteers and the percentage of time at pH of less than 4.0 was only around 5\%.\textsuperscript{78} Also, the pH of buffered solutions of high-buffer capacity (stronger buffers than usually used for \textit{in vitro} dissolution tests) infused into the upper jejunal of healthy subjects tended toward 6.0, regardless of the initial pH of the solution, 4.5 or 7.4.\textsuperscript{79} Therefore, the intestinal mucosa appears to be protected from acid loads, including physiological pulses of gastric content, and the duodenal microclimate pH seems to be kept around a set point by complex mechanisms based on the interplay of intestinal alkaline phosphatase, P2Y receptors, and cytosolic carbonic anhydrase, as well as on the role of Na\textsuperscript{+}/HCO\textsubscript{3}− co-transporters, as demonstrated in rats and mice, respectively.\textsuperscript{80,81} One of the few cases where intestinal pH is less well regulated is in cystic fibrosis patients, who have impaired pancreatic and biliary HCO\textsubscript{3}− secretion, resulting in lower and highly variable small intestine pH values.\textsuperscript{82,83} The lower intestinal pH in these patients might explain the 30\% reduction in ibuprofen $C_{max}$ observed in children with cystic fibrosis in comparison with healthy children.\textsuperscript{84}

In general though, given the above-mentioned protective mechanisms extant in most volunteers and patient groups, it seems highly unlikely that a weakly acid drug would markedly change the \textit{in vivo} small intestine microenvironment pH. Interestingly, although the final pH values of saturated solutions of ibuprofen are lowered by 2 units when solubility media of low-buffer capacity are used, as in the case of Hank’s balanced salt solution (buffer capacity = 1.6 mEq/L per pH unit), in which the pH changed from 7.56 to 5.52, a smaller reduction, around 1 pH unit, was observed \textit{in vivo}, after perfusing a segment of rat intestine with ibuprofen solutions using the same media. Moreover, pH values of perfusates changed toward a median of 6.5; even when a high-buffer capacity media, FeSSIF (~65 mEq/L per pH unit), was applied, the pH was significantly changed from 5.1 to 5.5.\textsuperscript{75} So, based on these results as well as on the fact that acid loads are neutralized before reaching jejunum,\textsuperscript{78} the following scenarios were assumed to be more realistic for describing the consequences of acidifying small intestine because of ibuprofen dissolution \textit{in vivo}: (1) a 2 pH unit drop in the duodenum only and (2) a 1 pH unit drop throughout small intestine.

Acidification of the duodenum because of dissolved ibuprofen does not translate into any PK or PD changes according to the simulations. Decreasing the small intestinal pH by 1 pH unit from the ADAM default pH values delayed $T_{max}$ by 15–25 min and decreased $C_{max}$ around 6\%. Nevertheless, neither the peak nor the extent of PD responses was affected. Mean $T_{min}$ and $T_{max}$ were delayed by 7 and 14 min, respectively, delays which are not clinically relevant. Although Tsume et al.,\textsuperscript{85} reported a similar pattern for AUC in their simulations, they observed a 25\% decrease in $C_{max}$ after reducing the pH by one unit throughout the small intestine.\textsuperscript{85} Different doses and intestine default pH values assumed in the simulations could account for the difference in the simulated magnitude of the decrease in $C_{max}$. But even a 25\% difference in $C_{max}$ does not seem to be clinically relevant, given the flat dose–response relationship of ibuprofen.

A 2 pH unit drop scenario throughout the small intestine resulted in a significant delay in both $T_{min}$ and $T_{max}$, around 40 and 85 min. However, this scenario is extreme, and it is only likely to occur in patients with specific gastrointestinal pathologies, such as cystic fibrosis or perhaps Zollinger–Ellison syndrome.
CONCLUSIONS

Because of the temporal disequilibrium between drug concentration in plasma and the analgesic and antipyretic effects elicited by ibuprofen, it seems that its PD response is relatively insensitive to changes in dose and consequently in the PK profile. Differences of up to around 30% in the PK response are translated into less than 7.5% and 9% changes in magnitude and extent of the PD responses measured, respectively, because of the flat concentration–response of ibuprofen. Simulations suggest that this relationship leads to the situation in which a generic drug product could still be considered therapeutically equivalent even though it is pharmaceutically bioinequivalent with respect to $C_{\text{max}}$. Accordingly, it seems safe to conclude that current BE criteria for ibuprofen is overly conservative.

Furthermore, ibuprofen behaves like BCS Class 1 drugs throughout the whole intestine, as it is highly soluble and permeable from the duodenum to the colon and its absorption seems to be largely controlled by a physiological variable, namely, gastric emptying. So, as long as the dissolution rate under intestinal conditions is faster than gastric emptying rate, formulation influences on the PK profile would be negligible and a BCS-based biowaiver decision would be feasible, as long as it is assured that critical excipients are qualitatively and quantitatively the same in T and R formulations.

Finally, reductions in small intestine pH commensurate with usual physiological variation do not seem to affect PD responses, and given that greater reductions in pH elicited by ibuprofen that have been observed in vitro and seem highly unlikely to occur in vivo, due to the protective neutralization mechanisms, using a dissolution medium with low-buffer capacity might result in differences in vitro that would not have any ramifications for therapy in clinical practice.

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