Simultaneous fitting of R- and S-ibuprofen plasma concentrations after oral administration of the racemate

Jörn Lötsch,¹ Uta Muth-Selbach,^{2,*} Irmgard Tegeder,¹ Kay Brune² & Gerd Geisslinger¹

¹Center of Pharmacology, Department of Clinical Pharmacology, Johann Wolfgang Goethe-University, Theodor Stern Kai 7, D-60590 Frankfurt and

²Department of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nürnberg, Universitätsstrasse 22, D-91054 Erlangen, Germany

Aims To assess the pharmacokinetic equivalence of two different formulations of ibuprofen lysinate with special focus on the expected effects.

Methods Sixteen healthy volunteers received cross-over ibuprofen lysinate as either one tablet of 400 mg ('test') or two tablets of 200 mg ('reference'). Ibuprofen plasma concentrations were followed up for 10 h. Bioequivalence was assessed by standard noncompartmental methods. Ibuprofen plasma concentrations were fitted with a model that took bioinversion of R- to S-ibuprofen into account.

Results Peak plasma concentrations of R- and S-ibuprofen were 18.1 and 20 $\mu\text{g ml}^{-1}$ (test), and 18.2 and 20 $\mu\text{g ml}^{-1}$ (reference). Areas under the plasma concentration vs time curves were 39.7 and 67.5 $\mu\text{g ml}^{-1} \text{ h}$ (test), and 41.1 and 68.2 $\mu\text{g ml}^{-1} \text{ h}$ (reference). Clearance of R-ibuprofen was 5.2 (test) and 5 l h^{-1} (reference). A specific plasma concentration was reached with the test formulation about 5 min later than with the reference. Parameters from compartmental modelling were (given for R- and then for S-ibuprofen): body clearance: 4.9 and 4.64 l h^{-1} , central volume of distribution: 2.8 and 4.1 l, intercompartment clearance: 5.1 and 5.45 l h^{-1} , peripheral volume of distribution: 4.1 and 5.2 l. The absorption rate constant was 1.52 h^{-1} , and the test but not the reference formulation had a lag time of 0.1 h. Simulations showed similarity between formulations of the expected effects except for a calculated delay of 6 min with the test formulation.

Conclusions Ibuprofen formulations were bioequivalent. The pharmacokinetic model may serve as a basis for future pharmacokinetic/pharmacodynamic calculations after administration of racemic ibuprofen.

Keywords: bioequivalence, ibuprofen, pharmacokinetics

Introduction

Ibuprofen is one of the most frequently used over-the-counter analgesics. Most commercial formulations come in tablets of 200 mg racemic ibuprofen. This contrasts with several reports that a dose of 400 mg racemic ibuprofen is probably more appropriate to

produce the desired analgesic effects [1, 3]. It is therefore reasonable to develop ibuprofen formulations with tablet doses of 400 mg. The present study was designed to compare the stereoselective pharmacokinetics of such a newly developed 400 mg tablet formulation with those of a standard formulation of 200 mg tablets. Both formulations contained ibuprofen as a lysinate that demonstrated less variable pharmacokinetics [4] and superior analgesic effects [5] when compared with older ibuprofen acid formulations. Based upon literature data concerning the relationship between ibuprofen pharmacokinetics and pharmacodynamics, the consequences of possible pharmacokinetic differences between formulations for the pharmacodynamic expected effects were estimated by simulation analyses.

Correspondence: Jörn Lötsch, M.D., Pharmazentrum Frankfurt, Department of Clinical Pharmacology, Johann Wolfgang Goethe-University Hospital, Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany. E-mail: jloetsch@em.uni-frankfurt.de

*Current address: University of Düsseldorf, Department of Anaesthesiology, Moorenstrasse 5, D-40225 Düsseldorf, Germany.

Received 2 May 2000, accepted 25 May 2001.

Methods

Subjects and study design

Sixteen healthy male volunteers, aged 22–36 years (mean 26.8, standard deviation 4.3 years) participated in this study. No violation of the usual exclusion criteria for studies with nonsteroidal anti-inflammatory drugs (NSAIDs) was found. The study protocol was approved by the University of Erlangen Ethics Review Committee and was conducted in accordance with the Declaration of Helsinki on biomedical research. All subjects provided written informed consent for participation in this study.

After having fasted overnight, the subjects took either one tablet of the test compound, a new ibuprofen lysinate formulation (684 mg ibuprofen lysinate = 400 mg ibuprofen) or two tablets of the commercially available reference product (Dolormin[®], 342 mg ibuprofen lysinate per tablet corresponding 200 mg ibuprofen, Woelm Pharma GmbH, Bad Honnef, Germany), together with 200 ml water. Food and fluids were withheld for a further 4 h. The washout period between both test phases in that cross-over study was at least 7 days.

Ibuprofen plasma concentrations

Venous blood samples were taken into potassium EDTA tubes prior to and at 0.167, 0.33, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 10 h after drug intake. Blood samples were immediately centrifuged and stored at -20°C with quality control samples until assay. Plasma R- and S-ibuprofen concentrations were measured by a validated stereoselective high performance liquid chromatography assay [6] employing an α_1 -acid glycoprotein column as a stationary phase. R- and S-ibuprofen for analytics were kindly provided by PAZ GmbH (Frankfurt, Germany). The optical purity of the enantiomers was greater than 98.5%. Calibration curves were prepared for the concentration range of $0.25\text{--}25\text{ }\mu\text{g ml}^{-1}$ of each enantiomer, over which the coefficient of variation was less than 5%.

Data analysis

Enantioselective noncompartmental pharmacokinetics were assessed with WinNonlin (standard, release 1.5, Pharsight Inc., Mountain View, CA, USA). Peak plasma concentrations, $C_{\text{max, observed}}$, and time to peak, $t_{\text{max, observed}}$, were noted directly. The lag-time, $t_{\text{lag, observed}}$, was defined as the time point before the time corresponding to the first concentration above the lower limit of quantification. Equivalence of the amount of ibuprofen absorbed was assessed by comparing the areas under the plasma concentration *vs* time curves (AUC)

between formulations. AUC was calculated using the linear trapezoidal rule for ascending concentrations and the log-trapezoidal rule for descending concentrations [7]. A regression line through the terminal linear segment of the log-transformed ibuprofen concentrations *vs* time curve was used for extrapolation to infinity. For R-ibuprofen, the slope of this line was equal to the terminal elimination rate constant, $\lambda_{z, R}$. Additional pharmacokinetic parameters of R-ibuprofen were clearance, CL_R , mean total residence time, MRT , and volume of distribution based on the terminal curve segment, V_{zR} , calculated using standard equations. Because bioavailability of ibuprofen lysinate can be regarded as $1(1.028 \pm 0.12)$ [8], it was possible to estimate clearance and volume of distribution from the present oral data. Pharmacokinetics of ibuprofen are complicated by the inversion of R- to S-ibuprofen [9]. The true amount of S-ibuprofen that entered the body consists of the sum of the administered S-ibuprofen and the S-ibuprofen formed by inversion of R-ibuprofen. Since the latter was not known, dose based parameters CL_S and V_{zS} could not be obtained for S-ibuprofen. For the same reason the MRTs have different interpretations for R- and S-ibuprofen. The mean total residence time being a sum of mean input time, MIT, and mean disposition residence time, MDRT [10] depends for R-ibuprofen on the absorption kinetics only, while for S-ibuprofen it depends in addition on the inversion kinetics of R-ibuprofen. Since the input of S-ibuprofen continues as long as R-ibuprofen is available, it cannot be excluded that the slope of the terminal segment of the plasma concentration *vs* time curve of S-ibuprofen is also confounded by R-ibuprofen inversion and does therefore not reflect $\lambda_{z, S}$.

Non-parametric 90%-confidence intervals [11] of the ratios of $C_{\text{max, observed}}$, and $AUC(0, \infty)$ between test and reference formulations were calculated. In addition, differences between R- and S-ibuprofen and between formulations in $C_{\text{max, observed}}$, $AUC(0, \infty)$, $t_{\text{max, observed}}$, MRT , and the percentage of the extrapolated AUC from the total AUC were assessed by means of analysis of variance for repeated measures (rm-ANOVA), with 'enantiomer' and 'formulation' as within-subject factors (degrees of freedom [d.f.] = 1,15). Since lag time was often zero and data did not meet the criteria for parametric analysis of variance, $t_{\text{lag, observed}}$ was compared between formulations by Wilcoxon signed rank tests, separately for R- and S-ibuprofen. In addition, CL_R , $\lambda_{z, R}$, $V_{z, R}$, and the individual ratios between the AUCs(0, ∞) of S- and R-ibuprofen were compared between formulations by means of *t*-tests. Statistics were done with SPSS 9.01 for Windows (SPSS Inc., Chicago, IL, USA; α level 0.05), except for the nonparametric 90%-confidence intervals that were calculated with Microsoft Excel 2000 for Windows (Microsoft Inc., Redmond, WA, USA).

Further analyses focused on the consequences of pharmacokinetic differences between formulations for the expected pharmacodynamic effects. In a first approach we assessed how quickly plasma ibuprofen concentrations rose to a defined plasma concentration between 5 and 40 $\mu\text{g ml}^{-1}$ (separated by steps of 5 $\mu\text{g ml}^{-1}$), and for how long such or higher concentrations were maintained. In addition to the enantiospecific data analysis, calculations were also performed for racemic ibuprofen in order to compare the results with values on racemic plasma ibuprofen being related to a specific therapeutic effect available from the literature [12]. The exact time when a specific concentration was reached was obtained using linear interpolation between two successive plasma concentrations in the case of ascending concentrations, and log-linear interpolation in the case of descending concentrations, in analogous fashion to the calculation of AUC [7]. Statistical significance of differences between formulations was assessed by means of rm-ANOVAS, with 'concentration' (d.f.=1, 15) and 'time/duration' (d.f.=7105) as within-subject factors.

A second approach to consequences of ibuprofen pharmacokinetics for the expected pharmacodynamic effects employed a link between the time profiles of plasma S-ibuprofen, the active enantiomer [13, 14], and the effects. An effect compartment linked to the plasma compartment was added to the pharmacokinetic model [15, 16]. Model development was simplified by the fact that presystemic inversion plays a minor role in the inversion of R- to S-ibuprofen in humans [17, 18], which made modelling of a first-pass effect unnecessary. It has to be mentioned that in case of slow intestinal absorption presystemic R- to S-ibuprofen inversion has been reported [19]. However, the same authors found much less presystemic inversion when a quickly absorbed ibuprofen solution had been administered [19]. Thus, with the presently administered fast-release ibuprofen the neglect of a possible presystemic inversion appears to be justified. The model (Figure 1) consists in principle of two separate models for R- and S-ibuprofen, each being composed of a central and a peripheral compartment, with inter-compartmental clearance Q . The respective enantiomer enters the central compartment from a depot compartment (consisting, in principle, of the tablet in the gastrointestinal tract) by a first-order process with rate constant k_a , and ibuprofen is eliminated into the environment from the central compartment. A fraction F_i of the clearance of R-ibuprofen consists of inversion clearance of R- to S-ibuprofen, $CL_{R,inv}$. The inversion was assigned to the central compartments what is compatible with the fact that most of the inversion takes place in the liver [20] while other sites play a minor role [21, 22]. The fraction F_i was set to 0.6 as reported from a previous study in a comparable study population [23]. Modelling was done

with NONMEM (version V 1.1, NONMEM Project Group, UCSF, San Francisco, CA, USA [24]). A naïve pooled data approach was chosen. Model building consisted of successive tests of the importance of every model parameter for the description of the data set, except for parameters of the effect compartment that was used only in the simulations. The final model was selected on the basis of the NONMEM objective function equal to minus two times the log likelihood, using the χ^2 approximation with the number of degrees of freedom equal to the difference between two models in the number of free parameters (α level 0.05). Equality of bioavailability between formulations was tested by introducing a bioavailability variable and setting it to 1 for the reference formulation but allowing it to be estimated for the test formulation. Differences between formulations in other model parameters were tested analogously, i.e. whether assigning to each model parameter a separate variable for each formulation improved the fit. An additive error model was chosen: $y=f(\phi, x)+\varepsilon$, where y is the dependent variable (i.e. plasma concentration), which is a function of known quantity x (i.e. time) and pharmacokinetic parameters ϕ [24] and ε is in the present naïve pooled data analysis a combination of interindividual, intraindividual inter-occasion, and residual variances. By definition, ε has mean of zero and variance of σ^2 . Proportional or additive and proportional error models consistently underestimated higher plasma concentrations. Calculations were performed using 'first order conditional estimation'. Ibuprofen concentrations at the hypothetical effect site were simulated by adding an effect compartment to the central compartment of S-ibuprofen (Figure 1). The simulations were based on k_{e0} values reported by Suri *et al.* [25] from the effects of ibuprofen on pain ratings ($k_{e0}=1.1\text{ h}^{-1}$) and pain related evoked cortical potentials ($k_{e0}=0.27\text{ h}^{-1}$) after electric tooth pulp stimulation. The effect was linked to the S-ibuprofen concentration at effect site by an $E_{\max}$ model, with $EC_{50}=8.71\text{ }\mu\text{g ml}^{-1}$ for the percent decrease in amplitudes of evoked potentials, and $EC_{50}=24.37\text{ }\mu\text{g ml}^{-1}$ for the percent decrease in pain ratings [25].

Results

All subjects completed the study and no adverse events were reported. Individual plasma concentrations of ibuprofen enantiomers after administration of the test and reference formulations are given in Figure 3. Concentration *vs* time profiles obtained with the test formulation, divided by those obtained with the reference formulation, showed no consistent pattern, indicating that differences between formulations were random (Figure 3, second from bottom). The results of statistical comparison of noncompartmental pharmacokinetic

parameters are listed in (Table 1). Non-parametric 90% confidence intervals of test/reference ratios for $C_{\max}$ were 0.92–1.07, with a median test/reference ratio of 1. The respective values of the $AUC(0, \infty)$ ratios were 0.96, 1.02 (median 0.98). Parametric statistics revealed

differences between R- and S-ibuprofen in $t_{\max}$, $C_{\max}$, $AUC(0, \infty)$, and the percentage of the extrapolated AUC from the total $AUC(0, \infty)$ (Table 1). No difference between formulations was found. However, $t_{\text{lag, observed}}$ for S-ibuprofen was significantly longer with the test than

$$\frac{dA(1)}{d(t-t_{\text{lag}})} = -k_a \cdot A(1)$$

$$\frac{dA(2)}{d(t-t_{\text{lag}})} = -k_a \cdot A(2)$$

$$\frac{dA(3)}{d(t-t_{\text{lag}})} = k_a \cdot A(1) - CL_R \cdot C(3) - Q_R \cdot C(3) + Q_R \cdot C(5)$$

$$\frac{dA(4)}{d(t-t_{\text{lag}})} = k_a \cdot A(2) - CL_S \cdot C(4) + CL_{R,inv} \cdot C(3) - Q_S \cdot C(4) + Q_S \cdot C(6)$$

$$\frac{dA(5)}{d(t-t_{\text{lag}})} = Q_R \cdot C(3) - Q_R \cdot C(5)$$

$$\frac{dA(6)}{d(t-t_{\text{lag}})} = Q_S \cdot C(4) - Q_S \cdot C(6)$$

$$\frac{dC(7)}{d(t-t_{\text{lag}})} = k_{e0} \cdot (C(4) - C(7))$$

$$C(3) = \frac{A(3)}{V(3)}, \quad C(4) = \frac{A(4)}{V(4)}, \quad C(5) = \frac{A(5)}{V(5)}, \quad C(6) = \frac{A(6)}{V(6)}, \quad C(7) \equiv A(7), \quad CL_{R,inv} = F_i \cdot CL_R$$

$$A(1) \text{ at times zero} = Dose_R, \quad A(2) \text{ at times zero} = Dose_S,$$

$$A(3), A(4), A(5), A(6), A(7), \text{ at times zero} = 0$$

with the reference formulation (Wilcoxon test: $P < 0.05$), although the difference was small. In contrast, that difference missed statistical significance for R-ibuprofen ($P = 0.101$).

The time to reach a specific concentration of racemic ibuprofen in plasma was longer with the test than with the reference formulation (Figure 2). This was supported by a significant interaction 'formulation by concentration' in the rm-ANOVA ($F = 2.59$, $P < 0.05$). The test formulation reached a defined plasma concentration 6 min later than the reference formulation, averaged among all concentrations. Higher concentrations were not reached in all subjects (Figure 2). No statistically significant difference between formulations was seen in the duration of a plasma concentration being equal or above a specific value (Figure 2). There was a roughly linear relationship between duration of maintenance of a plasma

concentration and the log plasma concentrations. Not surprisingly, a significant effect of the plasma concentration on the duration of its maintenance was seen in the rm-ANOVA ($F = 230.4$, $P < 0.001$).

The parameters of the final pharmacokinetic model of the ibuprofen plasma concentrations *vs* time course and their values are given in Table 2. The fit obtained in the naïve pooled data approach has been added to Figure 3. The time dependency of measured divided by predicted plasma concentrations is given at the bottom of Figure 3, showing that the fits were unbiased. By contrast, a model without peripheral compartments (analysis not shown) gave biased fits in terms of underestimation of concentrations at later sampling times. A lag-time of 5.8 min was assigned to the test formulation but not to the reference. Other model parameters did not differ between formulations. The 95% confidence interval of the estimate of the

Figure 1 Schematic presentation of the pharmacokinetic model of enantioselective ibuprofen plasma concentration *vs* time profiles after oral administration of racemic ibuprofen. The compartments are symbolized as squares, and the arrows between compartments symbolize the substance transfer, given in the equations at the arrows. The model consists of a system of differential equations, describing the change in amount of drug over time. Compartments 1, 3 and 5 belong to R-ibuprofen, compartments 2, 4 and 6 to S-ibuprofen. An effect compartment is linked to the central compartment of S-ibuprofen. By definition, no mass is transferred into the effect compartment, and the concentration, not the amount of drug at effect site is directly modelled. *A*: amount of drug, *C*: concentration, *CL*: body clearance, *Q*: intercompartmental clearance, *V*: the volume of distribution, k_{e0} : transfer rate constant from plasma to the effect site. The number of the compartment to which a parameter belongs is given in parentheses with the respective parameter. The fraction F_i of the total clearance of R-ibuprofen that forms the inversion clearance of R- to S-ibuprofen was set to 0.6 [23]. The differential equations describing the concentration *vs* time profile in the effect compartment derive as follows: The change of amount of S-ibuprofen over time in the effect compartment (compartment 7) can be initially described by

$$\frac{dA(7)}{d(t-t_{lag})} = V(7) \cdot \frac{C(7)}{d(t-t_{lag})} = V(4) \cdot k_{1e} \cdot C(4) - V(7) \cdot k_{e0} \cdot C(7),$$

where k_{1e} is the input rate constant into the effect compartment. Since the concentration in the effect compartment, $C(7)$, cannot be measured, its units are arbitrary and $V(7)$ can be chosen so that at steady state, SS, $C(4)_{SS}$ equals $C(7)_{SS}$. Then at steady state

$$V(4) \cdot k_{1e} \cdot C(4)_{SS} = V(7) \cdot k_{e0} \cdot C(7)_{SS}.$$

Dividing the equation by $C(4)_{SS} = C(7)_{SS}$,

$$V(4) \cdot k_{1e} = V(7) \cdot k_{e0},$$

and

$$V(7) = V(4) \cdot \frac{k_{1e}}{k_{e0}}.$$

Substituting that into the initial equation yields

$$\frac{dA(7)}{d(t-t_{lag})} = V(4) \cdot \frac{k_{1e}}{k_{e0}} \cdot \frac{C(7)}{d(t-t_{lag})} = V(4) \cdot k_{1e} \cdot C(4) - V(4) \cdot \frac{k_{1e}}{k_{e0}} \cdot k_{e0} \cdot C(7).$$

Dividing that equation by $V(4)$ and by k_{1e} and multiplying it by k_{e0} yields

$$\frac{C(7)}{d(t-t_{lag})} = k_{e0} \cdot C(4) - k_{e0} \cdot C(7)$$

[42, 43].

Table 1 Descriptive and inferential statistics of noncompartmental pharmacokinetic parameters obtained after administration of either one tablet of 400 mg racemic ibuprofen ('test') or two tablets of 200 mg racemic ibuprofen ('reference') to 12 healthy volunteers.

	Average (and s.d.) 1 × 400 mg 'Test'		2 × 200 mg 'Reference'		Parametric 95%-CI for differences test – reference	Statistical comparison Rm-ANOVA ANOVA-effect 'enantiomer'	t-test # ANOVA-effect 'formulation'	Difference of formulations
	R-ibuprofen	S-ibuprofen	R-ibuprofen	S-ibuprofen				
$t_{\text{lag, observed}}$ (h)	0.08 (0.12)	#	#	#	R # S #	#	#	#: Z = −1.64, P=0.101 #: Z = −2.08, P<0.05
$t_{\text{max, observed}}$ (h)	0.93 (0.12)	1 (0.57)	0.68 (0.3)	0.78 (0.33)	R −0.11, 0.6 S −0.15, 0.59	F=8.7, P=0.01	NS	–
$C_{\text{max, observed}}$ (μg ml ^{−1})	18.1 (3.6)	20 (2.9)	18.2 (3.7)	20 (3.4)	R −2.3, 2.1 S −1.7, 1.4	F=19.8, P<0.001	NS	–
AUC(0, ∞) (μg ml ^{−1} h)	39.7 (6.6)	67.5 (14.9)	41.1 (8.1)	68.2 (14.7)	R −6.2, 3.4 S −3.7, 2.4	F=84.9, P<0.001	NS	–
%AUC extrapolated	2.9 (2.1)	5.1 (2.2)	2.5 (1.7)	5.5 (2.9)	R −0.8, 1.7 S −1.4, 0.6	F=22.7, P<0.001	NS	–
AUC _S /AUC _R	1.7 (0.3)		1.7 (0.3)	–	– −0.1, 0.2	–	–	NS
CL (l h ^{−1})	5.2 (0.8)	–	5 (0.9)	–	R −0.4, 0.7	–	–	NS
λ_z (h ^{−1})	0.33 (0.08)	–	0.37 (0.1)	–	R −0.1, 0.02	–	–	NS
V_z (l)	16.3 (3.7)	–	14.7 (5.5)	–	R −1.9, −5	–	–	NS
MRT (h)	2.7 (0.66)	3.62 (0.68)	2.6 (0.49)	3.58 (0.68)	R −0.26, 0.45 S −0.26, 0.32	F=128.8, P<0.001	NS	–

Non-parametric 90% confidence intervals (CI) of test/reference ratios: C_{max} : 0.92, 1.07, AUC(0, ∞): 0.96, 1.02 (median 0.98). Note that interpretation of MRTs is different for R- and S-ibuprofen (see methods section). *n.s.* not significant. '–': Parameter not calculated/test not performed. #: Data of $t_{\text{lag, observed}}$ were often 0 and did not meet the criteria for parametric statistics. Parametric 95% CI on differences are therefore probably misleading. As a consequence, data of $t_{\text{lag, observed}}$ were analysed by means of Wilcoxon signed rank tests that revealed a significant difference between formulations for S-ibuprofen.

Figure 2 The time to reach a specific plasma concentration of racemic (left panels) and enantiomeric ibuprofen (central and right panels), and the duration during plasma concentrations were equal to or greater than a specific level (medians and interquartile ranges). Note the different scaling of the ordinates between racemic and enantiospecific ibuprofen in the lower panels. The number of subjects (out of 16) that reached a specific plasma concentration is given at the respective median concentrations. If a specific concentration was not reached in a subject, the time to reach that concentration was set to infinity. The median time for the reference formulation to reach $40 \mu\text{g ml}^{-1}$ was equal to infinity because that concentration was reached only in five subjects. Note that the x-axis at the top has a linear but at the bottom a logarithmic scale.

Figure 3 Observed plasma concentrations (dotted lines) of R- and S-ibuprofen after oral administration of 1×400 mg racemic ibuprofen ('test') and 2×200 mg racemic ibuprofen ('reference') to 16 healthy volunteers. The thick lines show the concentrations predicted by the pharmacokinetic model (Figure 1), using a naïve pooled data approach. The lower parts show plots of the quotients of individual ibuprofen plasma concentrations observed with of the test and the reference formulations (test/measured). In those plots, the lines at $y=1$ mark a quotient of 1 that would mean perfect equality of formulations. At the bottom the quotients of measured individual ibuprofen concentrations and the concentrations predicted by the pharmacokinetic model are shown, separately for test (lines) and reference (dotted lines) formulations. There was no bias in the predictions as indicated by the regression line superposed on the line of equality at $y=1$ (thick dashed lines).

Table 2 Parameters of the pharmacokinetic model of ibuprofen plasma concentration *vs* time profiles, obtained in a naïve pooled data fit of the data from the 12 study participants (see also Figure 1).

Parameter	Population central values (and % SEE)	
	R-ibuprofen	S-ibuprofen
CL ($l\ h^{-1}$)	4.9 (3.5%)	4.64 (4.9%)
Q ($l\ h^{-1}$)	2.8 (21.7%)	4.1 (18.7%)
V central (l)	5.1 (4.4%)	5.45 (4.7%)
V peripheral (l)	4.1 (9.7%)	5.2 (7.7%)
k_a (h^{-1})	1.52 (12.1%)	
t_{lag} (h)	'Test': 0.1 (29.9%), 'Reference': 0 Residual error	
σ^2	12.9	

The inversion clearance of R- to S-ibuprofen was set to 0.6 times the total clearance of R-ibuprofen [23]. % SEE: percent standard error of the parameter estimate, calculated as 100 times the ratio of the standard error of estimate (SEE) to the estimated parameter.

relative bioavailability of the test formulation included 1. The simulations (Figure 4) show that with the test formulation, the expected effects are identical for both formulations except for a approximately 5 min delay with the test formulation.

Discussion

The two formulations of ibuprofen lysinate met the criteria of bioequivalence, judged by the 90%-confidence intervals of the AUC(0, ∞) and C_{max} ratios. One tablet of 400 mg racemic ibuprofen of the test formulation produced almost identical plasma concentrations to two tablets of 200 mg ibuprofen in the reference formulation.

However, a specific plasma concentration was reached with the test formulation with a delay of approximately 6 min from the reference formulation. This applied also to the concentration of $30\ \mu g\ ml^{-1}$ reported by Laska *et al.* [12] as being the concentration of racemic ibuprofen above which no subject reported severe pain after tooth extraction. Simulation showed that the 5 min delay in the plasma concentrations with the test formulation is reflected in the time course of expected population central tendency of the effects. Since it is unlikely that a delay of 5 min is of any clinical relevance, the small pharmacokinetic differences between the two lysinate formulations of ibuprofen do not challenge the assumption of bioequivalence between formulations. This is especially true since the parameter estimates represent the central tendency in the population. Imagining interand intraindividual variance, the difference will probably not be noticed in the effects. Lag-time was not removed from the model because that would have been a violation of the predefined inclusion criteria for potential parameters of the final

pharmacokinetic model, i.e. significant improvement of the goodness of fit. In addition, it agrees closely with the delay seen in the descriptive data analysis. This adds further argument in favour of the decision not to remove lag-time from the final model. The apparent discrepancy between observations of lag-times with both formulations in the descriptive data analysis (Table 1) but only with the test formulation in the modelling analysis may be explained by goodness-of-fit criteria. Lag-time significantly improved the fit for the test formulation but it was too short to improve the fit compared with zero lag-time for the reference formulation.

More recently published k_{e0} values of 0.57 – $0.72\ h^{-1}$ obtained from pharmacokinetic-pharmacodynamic modelling of antipyretic ibuprofen effects in children [26] fall within the range 0.27 – $1.1\ h^{-1}$ [25] on which the present simulations were based. This consistency of the k_{e0} values in the literature increases the confidence in the simulated effect site concentrations. The link of the effects to S-ibuprofen only supports the currently accepted opinion that R-ibuprofen is inactive [27–30]. There are only a few indications in the literature that R-ibuprofen might contribute to the clinical effects after administration of racemic ibuprofen [31]. The most specific hypothesis refers to an inhibition of PGHS-2 induction by R-ibuprofenoyl-CoA thioester, an intermediate product in R-ibuprofen inversion [32]. However, currently available data are too vague give sufficient justification for incorporation of R-ibuprofen into the model. Since R-ibuprofen pharmacokinetics were as similar between the presently compared formulations as S-ibuprofen kinetics, future extension of the model by R-ibuprofen is unlikely to affect the conclusion of bioequivalence between formulations.

Simulation of the expected effects from the ibuprofen formulations based on a pharmacokinetic model that took the inversion of R- to S-ibuprofen into consideration. A direct estimation of F_i from the present data set was not possible because the true clearance of S-ibuprofen is unknown. Therefore, leaving F_i free to fit would have resulted in an unstable model: a low F_i could have been related to a low S-ibuprofen clearance as well as a high F_i to a high S-ibuprofen clearance, i.e. both F_i and CL_S would have been unidentifiable. There are several consistent reports of F_i of 0.56 and 0.6 [18], or 0.53 and 0.63 [23], estimated from urine and plasma data employing labelled S-ibuprofen, of 0.69 and 0.576 estimated from intravenous and oral administration of R-ibuprofen employing separate administration of pure S-ibuprofen [17] 0.63, 0.52, and 0.54 from studies employing separate and concomitant administration of ibuprofen enantiomers [33–35], and of 0.66 obtained by means of simulations based on S-/R-ibuprofen AUC(0, ∞) ratios [36]. Furthermore, the fraction inverted of R-ibuprofen has been

Figure 4 Time course of plasma concentrations of S-ibuprofen predicted by the pharmacokinetic model (Figure 1), and simulated times courses of analgesic effects, based on k_{e0} values of 1.1 h⁻¹ and 0.27 h⁻¹, and EC_{50} values of 24.37 and 8.71 µg ml⁻¹ for pain ratings and pain-related evoked potentials after electric tooth pulp stimulation, published by Suri *et al.* [25].

reported to be independent of the dose of racemic ibuprofen [37]. This consistency of the reported values of F_i in healthy volunteers justifies its use from the literature, unless data originate from subjects with liver disease where inversion is impaired [21], or when subjects were treated with clofibrate that increases the inversion of R- to S-ibuprofen [23]. The model presented here may also be used when direct information on S-ibuprofen pharmacokinetics is available from concomitant administration of labelled S-ibuprofen, making it possible directly to estimate F_i rather than incorporating a value from the literature.

The values of R-ibuprofen clearance estimated by the compartmental and noncompartmental analyses agree closely. Conversion of clearances and volumes into coefficients and exponents shows that this is also true for the values of $\lambda_{z,R}$, that are 0.36 h⁻¹ in the compartmental, and 0.33–0.37 h⁻¹ in the noncompartmental analysis. Furthermore, the lag time of 5.8 min estimated by modelling closely resembles the average delay of 6 min that was observed for the test formulation to reach a specific ibuprofen plasma concentration. Finally, both compartmental and noncompartmental analyses revealed equal bioavailability between test and reference formulations. In this respect, our data support the usefulness of the NONMEM approach to bioequivalence studies [38, 39], although those studies used population mixed effects modelling rather than a naïve pooled approach that was indicated for the present analysis by the lack of individual F_i values. The S-ibuprofen clearance of 4.64 l h⁻¹ corresponding to 77.3 ml min⁻¹ obtained with the modelling approach agrees with the value of 79.2 ml min⁻¹ reported by Hall *et al.* [18] after intravenous administration of (S)-d₄-ibuprofen to healthy volunteers. This indicates that assumptions of $F_i=0.6$ and a bioavailability of 1 were valid and did not result in an incorrect estimate of CL_S . By contrast, the 'clearance' value for S-ibuprofen

obtained in the noncompartmental analysis by $Doses/AUC_S$ was 3.3 l h⁻¹ = 55 ml min⁻¹ is in all probability incorrect because it based upon the administered S-ibuprofen dose of 200 mg and neglects the amount of S-ibuprofen resulting from R-ibuprofen inversion. In addition, the terminal half-lives of 1.94 and 2.12 h for R- and S-ibuprofen, respectively, obtained by reparameterization of volumes and clearances, resemble closely those of 1.74 and 1.77 h found after intravenous administration of either R- or S-ibuprofen in healthy men [17], or that of 1.96 h calculated after intravenous administration of (S)-d₄-ibuprofen in healthy volunteers [18]. The presented model as well as its parameters thus appear to be valid for the studied dose of 400 mg. Non-linearity caused by concurrent protein binding of S- and R-ibuprofen [37, 40] appears to be of little consequence when doses are below 600–800 mg, as suggested by the graphics in a paper assessing free and protein bound ibuprofen plasma concentrations [41].

In conclusion, we report a pharmacokinetic model that takes the inversion of R- to S-ibuprofen into account and allows for simultaneous fitting of enantiomeric ibuprofen plasma concentrations in subjects with normal liver function. The suitability of the model is emphasized by the close agreement of the S-ibuprofen clearance obtained after intravenous administration of S-ibuprofen [18]. It may serve to improve the mathematical description of plasma concentrations and effects *vs* time data obtained after administration of racemic ibuprofen. The present approach to comparative bioavailability estimation by looking at the effects to expect rather than at pure pharmacokinetic equality appears to be closer to the original goal of bioavailability studies, which is to ensure a similar therapeutic outcome after switching between pharmaceutical formulations. With the availability of data derived from PK/PD modelling for an increasing number of substances, the comparison of pharmacodynamic rather

than pharmacokinetic equivalence may be contemplated as future standard when comparing pharmaceutical formulations.

The study was supported by Woelm Pharma GmbH, Bad Honnef, Germany.

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