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Research Paper

Uncertainty Analysis in Pharmacokinetics and Pharmacodynamics: Application to Naratriptan

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Purpose. The aim of the study was to predict pain relief of migraine in patients following naratriptan oral (tablet) administration by using uncertainty analysis. The analysis was based on phase I pharmacokinetic naratriptan data, sumatriptan pharmacodynamic data, and naratriptan preclinical (animal) potency information, together with general knowledge as to how migraine affects oral absorption.
Methods. A previously developed pharmacokinetic (PK)/pharmacodynamic (PD) model for naratriptan disposition and effect was used. The uncertain parameters in the model, which were associated with absorption and scaling between first-in-class compound sumatriptan and naratriptan, were modeled using fuzzy sets theory. Global sensitivity analysis was then used to investigate the impact of each PK/PD parameter on the responses.
Results. Acknowledging parametric uncertainty did not improve prediction of the probability of pain relief. Global sensitivity analysis demonstrated that predictions were heavily influenced by interindividual variability in pharmacodynamics, as the dose response relationship was relatively insensitive to the pharmacokinetics.
Conclusions. To predict the probability of pain relief following oral (tablet) administration of naratriptan, a simple dose response, instead of the PK/PD model, would have yielded very similar predictions. The naratriptan PK/PD model may be improved by either refining the PD model or better still by specifying the interindividual error by additional data collecting with an improved design.

KEY WORDS: clinical trial simulation; fuzzy set theory; naratriptan; pharmacodynamics; pharmacokinetics; sensitivity analysis.

INTRODUCTION

Clinical trials simulation (CTS) is a powerful tool, increasingly employed by the pharmaceutical industry, which is used to predict and provide advice on various important issues, such as the optimal number of patients and optimal doses in a study (1–3). Simulation of clinical trials has established itself as a technique for knowledge synthesis and exploration of possible clinical trial results based on a mathematical and stochastic model of the trial, including submodels of drug action and disease process (4,5). Any clinical trial simulation exercise is based on three major components: (1) a set of models, (2) a clinical trial protocol, and (3) adequate resources (6). The outcome of CTS is quite heavily dependent on the quality of the model(s) used. Any attempt to improve the pharmacokinetic (PK)/pharmacodynamic (PD) model by incorporating the existing parameter variability and uncertainty and by investigating structural uncertainty will have a potentially positive effect on the resulting knowledge of the simulated trial.

Because of pressure on drug development programs, information is accumulated at an uneven rate, which often results in better quantitative characterization for some processes and parameters and semiquantitative, even vague information for others. Quantitatively well-defined parameters can be specified as random variables, characterized by probability density functions (pdfs), whereas vague parameters can be defined as fuzzy numbers in the fuzzy sets theory framework (7). Predictions made from phase I and II clinical trials will benefit from formal incorporation of all available, yet heterogeneous, information. Additionally to parametric uncertainty, there is also uncertainty related to the structure of the model. Structural uncertainty, which concerns model structure assumptions, parametric interactions, and the like, can be studied via global sensitivity analysis (GSA) (8). Phases I and II are critical stages of the drug development process, where the therapeutic effectiveness of a new drug is assessed and a decision whether to carry on with its further

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development is made. It would be useful if, at these stages, a reliable prediction of the drug candidate's probability of therapeutic success could be made.

Sumatriptan and naratriptan are selective $5HT_{1B/1D}$ receptor agonists used for the treatment of acute migraine. Sumatriptan was the first triptan to be developed (9,10). Sumatriptan is clinically available as an injection for subcutaneous administration and as a tablet for oral administration. The mean subcutaneous bioavailability is 95% compared to 14% for the oral tablet (11). Sumatriptan was the first in line, followed by naratriptan, which was chemically designed to have better oral bioavailability.

The current study follows from a previous investigation (6), where the feasibility and utility of model-based clinical trial simulations and design for the phase II development of naratriptan with effect measured on a categorical scale were explored. In their study, Nestorov *et al.* used mixed effects modeling to identify the pharmacokinetic model, estimate its parameters from phase I data, predict the probability of pain relief following naratriptan oral (tablet) administration, as well as suggest optimal designs for phase II and III studies. Because of lack of experimental data, parameter estimates connected with absorption, such as oral bioavailability, absorption rate, and lag time, were quite uncertain. In their study, Nestorov *et al.* assumed that the uncertain parameters were fixed, which left possible alternatives unexplored and may have influenced predictions.

This study aims to predict pain relief of migraine in patients following naratriptan oral (tablet) administration by using fuzzy sets theory, which is considered to be well equipped to handle uncertain information (12). An attempt was made to improve the modeling by performing uncertainty analysis in two stages: (1) representing the uncertain parameters by fuzzy numbers and incorporating the heterogeneous information to predict probability of pain relief following naratriptan oral administration and (2) global sensitivity analysis of the naratriptan PK/PD model.

METHODOLOGY

The basic assumption in the current study was that the available sumatriptan PD information can be integrated together with preclinical, early clinical (phase I) naratriptan (iv, subcutaneous and oral solution) information and general knowledge regarding the effect of migraine in patients. The PD assumption described above is based on the fact that naratriptan and sumatriptan are chemically similar and have a similar (if not identical) mechanism of action.

Data

Data used for specification of the naratriptan PK/PD model following oral (tablet) administration came from several sources.

Naratriptan Phase I Study

The first source was plasma profiles from naratriptan phase I studies following intravenous, subcutaneous, and oral (solution) routes of administration of naratriptan from a total of 26 healthy male volunteers.

Naratriptan Phase II Data

Phase IIa data derived from subcutaneous administration of placebo and naratriptan administration to 400 patients (mostly female), including 33 patients on active treatment for which there were both pharmacokinetic and headache score data. The subcutaneous doses of naratriptan were 0, 0.5, 1, 2.5, 5, and 10 mg. There were 63, 60, 55, 42, 34, and 34 patients in each dose group, respectively. The PK sampling times were 0, 0.167, 0.333, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h.

Preclinical Sumatriptan Data

To allow scaling between naratriptan and sumatriptan, published preclinical sumatriptan data were used (13,14). These studies showed that naratriptan is 2- to 6-fold more potent than sumatriptan, but they have approximately the same maximum effect.

Sumatriptan Pharmacodynamic Data

Data from a study investigating the dose-response relationship for sumatriptan, in terms of headache relief, after administration of nasal sumatriptan (15) were used. These data were also employed in the original paper (6).

Naratriptan PK/PD Model Following Subcutaneous and Oral Administration, Parameter Estimates, and Modeling Assumptions

Naratriptan Pharmacokinetic/Pharmacodynamic Model

Analysis conducted using a population pharmacokinetic approach (NONMEM ver. V) revealed that a two-compartmental open model for the disposition kinetics with a first-order absorption model for the subcutaneous and oral routes provided the best fit to the naratriptan data (6). A schematic representation of the adopted model is given in Fig. 1, and the parameter estimates are listed in Tables I and II. The pharmacodynamic model was based on the clinical end point of naratriptan; migraine pain relief/severity with a conservative and a fixed naratriptan/sumatriptan ratio of 2 was used to scale the potency parameter in the sumatriptan pharmacodynamic model (6). Pain relief was measured on a five-point ordered categorical scale defined as follows: 0 = no pain relief, 1 = mild pain relief, 2 = moderate pain relief, 3 = considerable pain relief, and 4 = total pain relief. Pain severity was measured on a four-point ordinal scale with 0 = no pain, 1 = mild pain, 2 = moderate pain, and 3 = severe pain. Regulatory requirements define a measure of "success" of a drug as the ability to reduce pain severity or increase pain relief at 2 h "significantly." For pain severity, a success is a reduction from categories 3 or 2 to 1 or 0, and for pain relief, it is an increase from categories 0, 1, or 2 to 3 or 4. Because of the regulatory requirements of significant pain relief, only the cut point corresponding to 1 was considered necessary in Ref. (6). Simulations based on dichotomizing the categorical responses into pain relief (success) and no pain relief (failure) were performed. The response was modeled by a binary logistic model for dichotomization, pain relief/no pain relief as defined in Eq. (1) (6). The first line of Eq. (1)

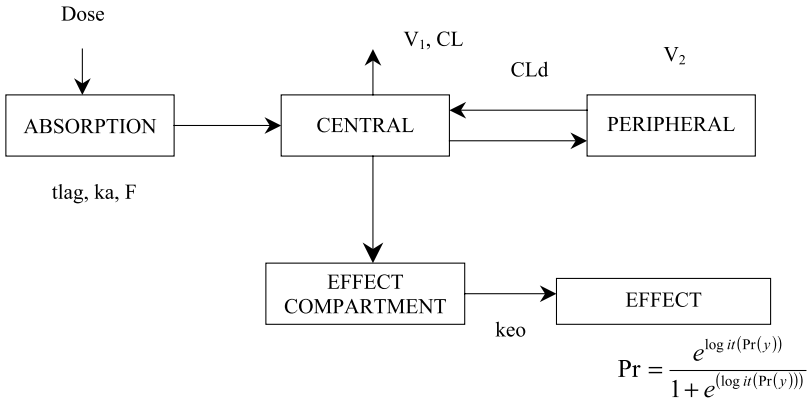

Fig. 1. Two-compartment open-body disposition model for naratriptan kinetics following oral (tablet) administration, linked to the effect compartment.

corresponds to a baseline pain severity score of 2. If the patient reported a pain severity score of 3 after the baseline measurement, then this is recorded as 2 as only the decrease of pain severity is of interest. The second line corresponds to a baseline pain severity score of 3. Hence, the first part of the model has cut points 0 and 1, whereas the second part has cut points of 0, 1, and 2. This is defined by conditioning on the baseline severity variable score_0. The drug effect is defined as an "Emax"-type model where C_e [Eq. (1)] is the concentration of the drug at the hypothetical effect site.

$$\log it(\Pr(Y_{ij}=1|\text{score}_0)) = \begin{cases} \theta_1 + \theta_4 \log(\text{time}_{ij}) + \dfrac{\theta_5 C_{eij}}{\theta_6 + C_{eij}} \\ \quad +\eta_i, \ \text{score}_0 = 2 \\ \phi_1 + \phi_5 \log(\text{time}_{ij}) + \dfrac{\phi_6 C_{eij}}{\phi_7 + C_{eij}} \\ \quad +\xi_i, \ \text{score}_0 = 3 \end{cases} \quad (1)$$

The naratriptan PD model is obtained from the sumatriptan PD model by scaling the affinity parameters θ_6 and ϕ_7 by the reported relative potency factor (14) between naratriptan and sumatriptan (see Table III).

Assumptions Made for the Uncertain Parameters

Because of lack of data for their estimation, the parameters related to the absorption of the oral form and the potency factor are quite uncertain. Nestorov *et al.* (6) fixed their values on the basis of previous sumatriptan information and general knowledge about the effect of migraine. It is known that migraine is usually accompanied by delayed gastric emptying, which would increase the absorption lag time (16). Nestorov *et al.* (6) reported that the experimental data showed evidence of significant variability in the bioavailability of naratriptan, the likely coefficient of variation being in the range of 40–60%. Yet, the employed population model for the PK/PD model was unable to estimate this variability, possibly assigning it to other parameters. The same consideration applies to the model rate constant keo, which represents the PD delay in the system between plasma concentration and response. To study the influence of the uncertainties on the power of the trial, Nestorov *et al.* simulated clinical trials with different coefficients of variation (0, 30, and 60%) for the uncertain parameters, concluding that they did not have a great impact on the power of the clinical trial.

Model Simulations

Three sets of analysis were carried out. Firstly, a previously identified naratriptan pharmacokinetic model following subcutaneous administration (6), integrating preclinical and phase I data, was used to perform fuzzy simulations for predicting the phase IIa data. This required the use of phase I naratriptan pharmacokinetic data as well as uncertain information about the parameters concerned with the absorption process. In this analysis, the emphasis was on the following: (1) specifying fuzzy numbers for the uncertain parameters; (2) checking the feasibility of the necessary transformation from pdfs (see section below), representing the quantitatively rich, estimated parameters as fuzzy numbers; as well as (3) evaluating the predictive ability of the

Table I. Pharmacokinetic Parameters Obtained from Phase I Data

Pharmacokinetic parameters	Mean (SD[a])
CL (L h^{-1})	22.7 (6.33)
V_1 (L)	17.2 (3.56)
V_2 (L)	147 (38.7)
CL_d (L h^{-1})	154 (39.3)
ka (h^{-1})	4.01 (2.57)
F	0.96 (n.a.)

[a] Intersubject variance.

Table II. Parameter Estimates Obtained from Phase IIa Data

Parameters	Mean (SD[a])
tlag (h)	0.35 (0.35)
CL (L h^{-1})	17.9 (7.57)
V_1 (L)	23.0 (20.1)
V_2 (L)	98.4 (47.0)
CL_d (L h^{-1})	101.4 (36.8)
ka (h^{-1})	0.85 (0.28)
F	0.48 (n.a)
keo (h^{-1})	0.85 (n.a.)

[a] Intersubject variance.

Table III. Scaled Naratriptan Pharmacodynamic Model [Eq. (1)] Parameters

	Model parameter	Estimate (SE)
Model conditional on baseline score = 2		
Baseline score	θ_1	−2.06
Placebo/time effect	θ_4	1.93
Maximum effect	θ_5	3.96
Ce50 (ng/mL)	θ_6	3.51/ Potency factor
Intersubject variability	ω_η^2	17.7 (2.14)
Effect site equil. rate const.	keo	0.78 (0.49)
Model conditional on baseline score = 3		
Baseline score	ϕ_1	−4.38
Placebo/time effect	ϕ_5	2.32
Maximum effect	ϕ_6	9.85
Ce50 (ng/mL)	ϕ_7	45.9/ Potency factor
Intersubject variability	ω_ξ^2	19 (2.42)
Effect site equil. rate const	keo	2.04 (1.04)

integrated model constructed from heterogeneous sources of information and general knowledge. Secondly, the two-compartment open model developed for naratriptan kinetics following oral (tablet) administration, together with the estimated parameters and the specified fuzzy numbers for the uncertain parameters, was used to predict the probability of pain relief in patients. Thirdly, global sensitivity analysis was undertaken on three of the main outputs: plasma concentration, effect site concentration, and probability of pain relief. In this analysis, emphasis was on apportioning the output variance caused by the variance of the different factors, i.e., PK/PD parameters. This allowed investigation of the factors contributing to the variance of the output temporal profiles.

Fuzzy Numbers Assigned to the Uncertain Parameters

Fuzzy sets theory (12) incorporates measures of uncertainty and variability in variables (e.g., model parameters) by representing them as fuzzy sets or fuzzy numbers. A fuzzy number is defined by an interval (support) and a membership function. The membership function is often derived from data, or alternatively, it could be based on the belief of the investigator(s) (or experts) for the level of certainty of these possible values. Alpha cuts are computationally convenient forms of representation of fuzzy numbers, and any fuzzy number can be represented as a union of its α-*cuts* [for more details, see Ref. (17)].

The bioavailability of naratriptan oral tablets in patients during a migraine attack should be less compared with migraine-free healthy volunteers, receiving oral solution (18). This decrease is reported (18) to be about 20%, and the intersubject variability is likely to be increased. Based on this knowledge, the most certain values (membership function 1) for *F* are between 40 and 60%. To allow for every possibility, thus reflecting the underlying uncertainty, a bell-shaped fuzzy number was specified (Fig. 2a) with a very steep exponential decrement from 40 to 0% and from 60 to 100%. Thus, at α-cut equal to 0, the bioavailability can assume any value in the closed interval [0, 1], which is a physiologically justified assumption. Steep as opposed to gradual slopes of the curves represents our belief that the naratriptan bioavailability is between 40 and 60%. The asymmetric bell-shaped membership function bears on the fact that there were some experiments quoting a bioavailability of around 70%, whereas none were in the range [0, 40%]. Thus, a bioavailability of 70% is a possibility at lower levels of certainty. There is evidence that a migraine attack impairs drug absorption severely (19,20). The fuzzy number for the absorption rate constant (ka) is based on the knowledge that its value should be decreased even more than bioavailability by the migraine attack, and its variability is also likely to be increased (Fig. 2b). The most typical range of ka is assumed to be between 0.55 and 0.65 h^{-1}, and between 0.5 and 0.7 h^{-1} with the lowest certainty. A trapezoidal fuzzy number was specified to reflect two facts: (1) our inability to distinguish between the values in the closed interval [0.55, 0.65] and (2) the assumption that naratriptan ka is not less than 0.5 and not more than 0.7. The fuzzy number for the lag time (Fig. 2c) is based on that observed for healthy volunteers and our belief that its most typical values are within the closed interval [0.3, 0.6]. The fuzzy number for the potency factor (Fig. 2d) is based on a reported study (14) that the pharmacological profile of naratriptan is very similar to that of sumatriptan, but naratriptan is at least "two to three times" and even possibly up to six times more potent. A bell-shaped fuzzy number is assumed for this parameter with most typical values in the closed interval [2, 3] and with a steep decrement to the closed interval [0, 6] as less certain, yet possible values for the potency factor. The asymmetric shape, assigning more certainty to values between 3 and 6 than to values in the interval [0, 2], bears on two facts: (1) naratriptan was chemically designed to be more potent than sumatriptan; (2) a number of experiments reported (14) give a potency factor with values in the closed interval [3, 6], whereas no studies have reported a potency factor in the [0, 2] closed interval. The fuzzy numbers for the equilibration rate constant (keo) for the two pain scores 2 and 3 are given in Fig. 2e. Based on reported probability density functions (6), derived from a reasonably large number of observations, the cores [interval of values with highest certainty; see Ref. (17)] of the triangular fuzzy numbers are the reported mean values and their supports are ±2σ, representing 95.4% of the underlying probability density functions.

Transformations and Fuzzy Simulations

Normalization is a commonly used technique for probability–possibility transformation.

It preserves the form of the underlying probability density function and ensures that the core of the resulting fuzzy number is always one (21).

The normal or Gauss distribution in probability is defined by

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{x-mean}{\sigma}\right)^2}, \quad -\infty < x < \infty \qquad (2)$$

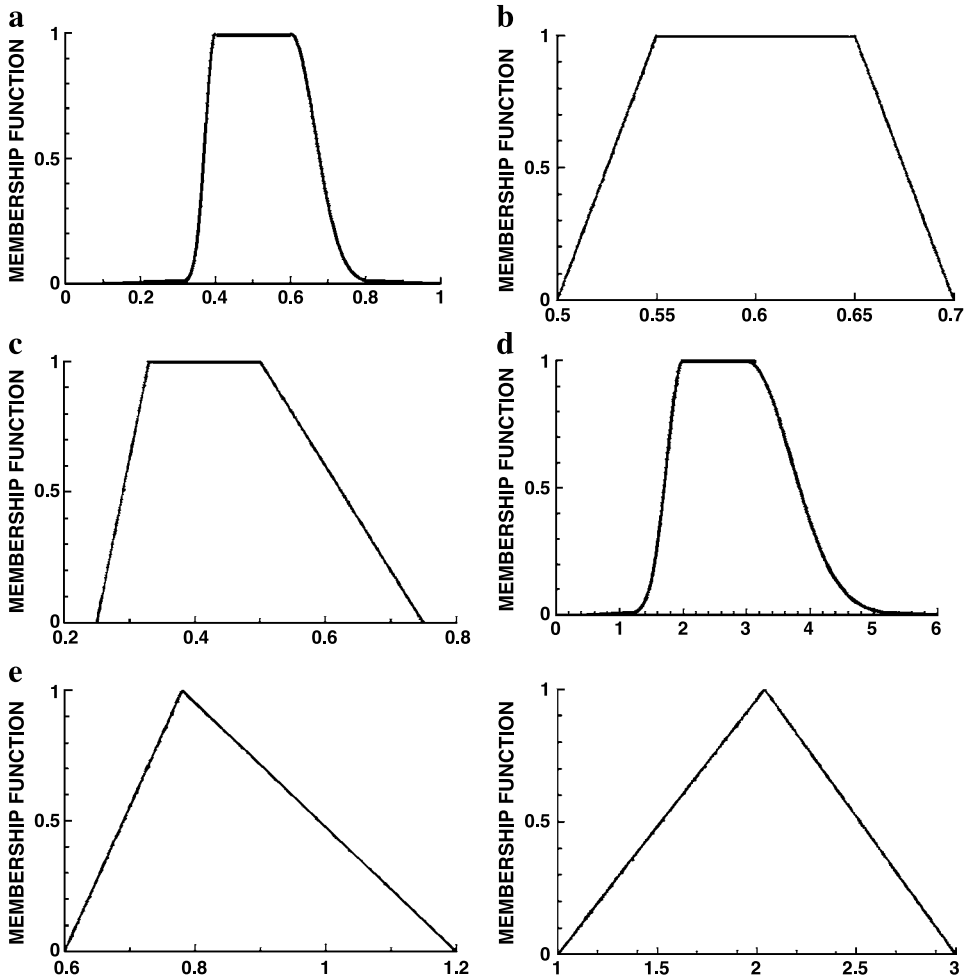

Fig. 2. Assigned fuzzy numbers following oral administration of naratriptan for (a) bioavailability, (b) the absorption rate constant, (c) lag time, (d) the potency factor between sumatriptan and naratriptan, and (e) the equilibration rate constant, keo, for different pain scores: pain score 2 (left panel) and pain score 3 (right panel).

where mean is the mean and σ is the standard deviation. The distribution is symmetric with respect to mean. As σ gets smaller, the peak becomes higher. For $\sigma = \frac{1}{\sqrt{2\pi}}$, the peak is (mean, 1). To construct a fuzzy number from a normal distribution $\sigma = \frac{1}{\sqrt{2\pi}}$, which gives a function with maximum 1, Eq. (3) is used

$$\alpha = F_A(x) = e^{-\pi(x-mean)^2}, \quad x \in (-\infty, \infty) \quad \alpha \in [0, 1] \qquad (3)$$

where mean is a parameter that determines the width of $F_A(x)$.

The same reasoning is used for transforming a lognormal pdf to a fuzzy number. To propagate the existing parametric variability and uncertainty through the model, all parameters have to be in the same type of representation, e.g., either random variables or fuzzy numbers. The heterogeneous PK/PD parameters need to be brought into one and the same form of uncertainty and variability representation. It was decided to transform the pdfs into fuzzy numbers according to the normalization criterion above. Although this results in some information loss (22), as pdfs are considered to be full quantitative characterizations of the parameters, no artificial information is introduced and the shape and symmetry of the underlying pdfs are preserved in the transformed fuzzy numbers. Representatives of the resulting transformed fuzzy numbers from pdfs are given in Fig. 3. Following the transformations, all PK/PD parameters are represented as fuzzy numbers. Fuzzy simulations to obtain plasma predictions, following naratriptan subcutaneous administration, were performed with an α-*cut* step size of 0.1. This was appropriate as only several levels of certainty were important. However, when predicting effect site concentration and probability of pain relief, a smaller step size (0.01) was chosen to allow interpolations among the predicted levels of certainty for the probability of pain relief.

Global Sensitivity Analysis of Naratriptan Pharmacokinetic/Pharmacodynamic Model

Global sensitivity analysis was performed on the naratriptan PK/PD model to achieve two aims: (1) to investigate whether the model resembles the system and fulfills our expectations and (2) to characterize quantitatively the contribution of each factor to the variance of plasma and effect site concentrations and also the variance of the probability of pain relief. Global sensitivity analysis involves

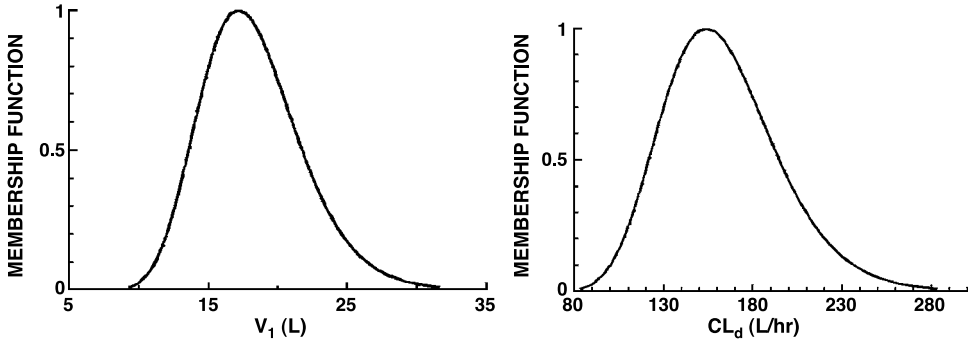

Fig. 3. Transformed pharmacokinetic parameters as fuzzy numbers.

five stages: pdf characterization of factors, assigning frequencies, defining search curves, evaluating the PK/PD model, and estimating sensitivity indices. Extended Fourier amplitude sensitivity test (FAST) techniques were implemented to investigate the influence of the variation in factors on the variance of predicted plasma and effect site concentrations, as well as on the predicted probability of pain relief. A factor with a sensitivity index of 0 is interpreted as not contributing at all to the variance of the output, whereas a factor with a sensitivity index of 1 is responsible for all of the variance of the output. First-order sensitivity indices (FOSI) represent only the contribution of the variance of the factors to the variance of the output. All FOSI do not have to sum up to 1, as factor-to-factor interactions of any order are not accounted for. The total order sensitivity index (TOSI) of a factor comprises the output variance due to variance of the factor plus interactions of this factor with the other factors of any order. A specification of naratriptan PK/PD factors (as specified below) together with the number of model evaluations performed follows.

Naratriptan Pharmacokinetic/Pharmacodynamic Factors

A factor is defined as a parameter, which is not held fixed, and its uncertainty and variability are acknowledged by regarding it as a random variable with a specific probability distribution function. Factors in our analysis are bioavailability, absorption rate constant, clearance, volume of the first (central) compartment, volume of the second (peripheral) compartment, distributional clearance, equilibration rate constant, potency factor, and intersubject variability. These factors, together with their respective assumed distributions, are given in Table IV.

It was decided that the factors that have been estimated previously by data fitting (6) would keep their pdf, whereas the factors that were quite uncertain were assigned uniform distributions within the closed interval of most certain, i.e., membership function 1, values. The PD parameters, kept fixed at their nominal values, for the GSA are listed in Table IV.

Number of Pharmacokinetic/Pharmacodynamic Model Evaluations

Two different values for the number of simulations were used in the PK/PD model GSA analysis. Initially, first- and total-order sensitivity indices were evaluated by performing a large number of simulations (34,803). Subsequently, a much smaller number of simulations (945) calculated with a different maximum frequency [for more details, see Ref. (8)] were performed and yielded sensitivity indices similar to that calculated by the much larger number of simulations. This finding supports those of Ref. (23), who showed that the size of the interference and aliasing errors depend on the both the number of factors and simulations employed. No benefit is gained by employing more simulations than needed to achieve distinguishability among the factors. Hence, only the results from the smaller number of simulations (945) are reported in this current study.

Software

Several specialized software packages were used to conduct this study. SimLab 1.1 (24) has several procedures

Table IV. Naratriptan Pharmacokinetic/Pharmacodynamic Model Factors with Their Respective Assumed Distributions Used in the Global Sensitivity Analysis

Factors	Probability density function type	Values
F	Uniform	0.4–0.6[a]
ka (h^{-1})	Uniform	0.55–0.65[a]
CL (L h^{-1})	Lognormal	17.9 (7.57)[b]
V_1 (L)	Lognormal	23.0 (20.1)[b]
V_2 (L)	Lognormal	98.4 (47.0)[b]
CL_d (L h^{-1})	Lognormal	101.4 (36.8)[b]
keo (h^{-1})	Uniform	0.6–1.2[a]
Potency factor	Uniform	2–3[a]
Intersubject variability	Normal	17.7 (2.14)[b,c]
		19.0 (2.42)[c]
tlag (h)	Fixed	0.415
Baseline score θ_1	Fixed	−2.06
Placebo/time effect θ_4	Fixed	1.93
Maximum effect θ_5	Fixed	3.96
Ce50 (ng/ml) θ_6	Fixed	3.51/Potency factor[d]
Baseline score ϕ_1	Fixed	−4.38
Placebo/time effect ϕ_5	Fixed	2.32
Maximum effect ϕ_6	Fixed	9.85
Ce50 (ng/ml) ϕ_7	Fixed	45.9/Potency factor[d]

[a] Lower–upper bounds.
[b] Mean (SD).
[c] Either of these two values is used depending on the pain score.
[d] Value sampled from the specified probability density function for the current simulation.

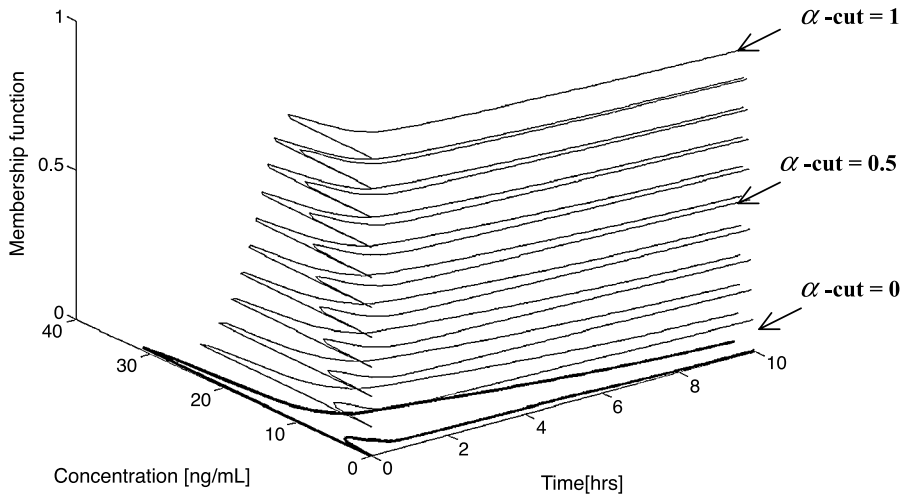

Fig. 4. Simulated fuzzy plasma concentration-time prediction following subcutaneous administration. The predicted concentration bands at different levels of certainty, i.e., α-*cuts*, are shown. Time (h) is given on the *x*-axis, predicted concentration (ng/mL) on the *y*-axis, and membership function on the *z*-axis. The predicted concentration at the lowest level of certainty is shown with a solid line to indicate that this α-*cut* (α-*cut* 0) was used later on for comparison with experimental data.

for global sensitivity analysis, one of which is the extended FAST method. SimLab 1.1 was used to sample from the PK/PD model factors (Table III). The sampled sets were imported into MATLAB 6, where they were assigned to the coded PK/PD model and simulations were performed with the sampled factors, solving the PK/PD model. Stanford Graphics 3.0c and MATLAB 6.1 are used to graphically represent the results.

RESULTS

Modeling the Naratriptan Data from Phase I Studies

Fuzzy simulations were performed to predict naratriptan plasma concentrations following subcutaneous administrations (Fig. 4). These predictions were compared with the available data from the naratriptan phase I study. As expected, they show good agreement with the data from which they were derived (Fig. 5a). The simulated profiles at low certainty level (solid curves) predict quite well the phase I data, which were used to estimate the PK parameters. This was expected and confirmed the feasibility of the various transformations employed. Using these transformed parameters, predictions were made for naratriptan in the phase IIa study following subcutaneous administration. The simulated profiles were plotted for comparison with patient data obtained from the actual phase IIa studies (Fig. 5b). The naratriptan model based on phase I data underpredicted the phase IIa data. The reason for this discrepancy is not known. Differences in the pharmacokinetics between healthy volunteers (predominantly male) and migraine patients (mainly female), i.e., gender differences, might explain some of this discrepancy, although this effect has not been previously reported (10).

Simulating Naratriptan Pharmacokinetics/ Pharmacodynamics Following Oral Administration

Trials after oral administration with doses 0, 0.5, 1, 2.5, 5, and 10 mg were simulated. Predicted effect site concentrations at different doses at a fixed time (2 h; specified by the regulatory

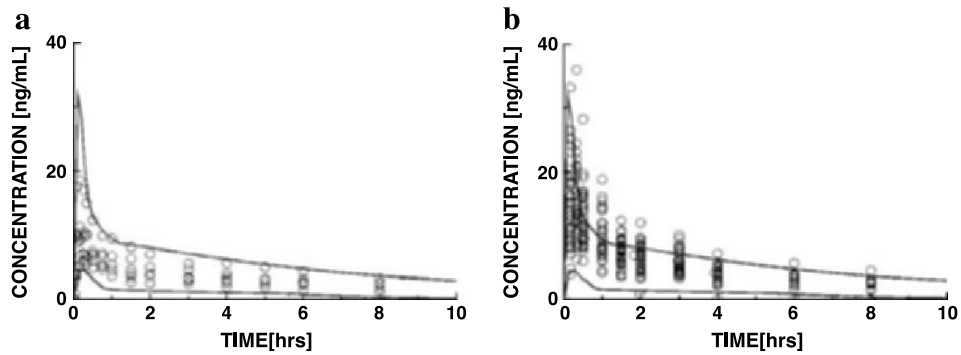

Fig. 5. Naratriptan predicted plasma concentration-time profiles at α-*cut* 0 (solid curves) following subcutaneous administration (a) prediction for phase I (healthy volunteers); (b) prediction for phase IIa (patients) study; data (circles).

authorities, see Methodology) are shown in Fig. 6a. With no drug present, a singleton fuzzy number for the effect site concentration was predicted for the placebo. As expected, the effect site concentration increased with the increment in dose. The probability of pain relief at different doses at 2 and 4 h is given in Fig. 6b and c, respectively. A fuzzy number was predicted for the probability of pain relief at each dose. These fuzzy numbers, representing the probability of pain relief for each dose, were defuzzified using the centroid method (7), given by Eq. (4). This was performed to facilitate better appreciation and easier comparison with the experimental data.

$$z^* = \frac{\int \mu_C(z) z dz}{\int \mu_C(z) dz} \tag{4}$$

where z^* is the defuzzified value and μ_C is the membership function.

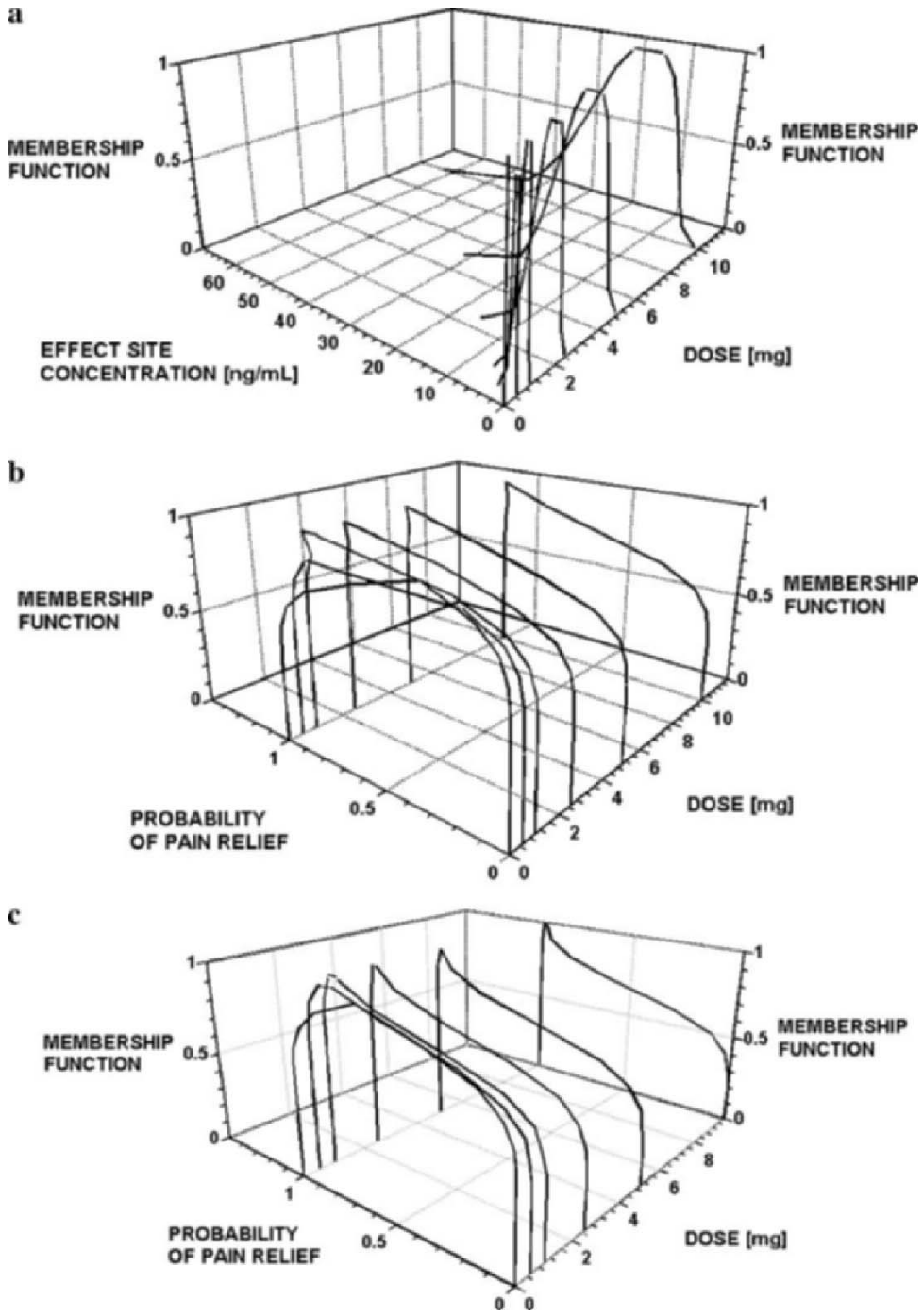

Fig. 6. (a) Predicted concentration profile at the effect compartment at 2 h. At each dose, a fuzzy number was predicted for the effect site concentration; (b) predicted probability of pain relief at 2 h; (c) predicted probability of pain relief at 4 h. At each dose, a different fuzzy number was predicted for the probability of pain relief.

The predicted probability of pain relief at 4 h, compared with two dose ranging studies reported by Refs. (10) and (25), is given in Fig. 7a. The predicted probability of pain relief at 2 h was also compared with another reported literature study (13) and is given in Fig. 7b. The observed probability of pain relief associated with the placebo, as reported (13), was also plotted with the predicted placebo probability of pain relief (Fig. 7c). The simulated probability of pain relief at 4 h was in good agreement with the reported data except for the placebo and low doses where overprediction occurs. The predictions were not encouraging, however, as the overprediction is at doses with expected therapeutic application, i.e., between 1 and 5 mg. When comparing predicted probability of pain relief at 2 h with the findings of Ref. (13) (Fig. 7b), the data are underpredicted by the model at every dose except at placebo. A likely reason for overprediction (Fig. 7a) and underprediction (Fig. 7b) is the use of a relatively insensitive pharmacodynamic model, which can be concluded from the placebo study (Fig. 7c). To substantiate this suggestion, global sensitivity analysis was performed.

Global Sensitivity Analysis of the Naratriptan Pharmacokinetic/Pharmacodynamic Model

The variance of the plasma concentration was decomposed into the contribution of the different factors, these being bioavailability, absorption rate constant, clearance, distributional clearance, and the volumes of the first and second compartments. The influence of the bioavailability (F), with pdfs as given in Table IV, on the variance of the predicted plasma concentration at different doses with respect to time is shown in Fig. 8a. The surface shadowing is performed according to the sensitivity indices calculated using the extended FAST method. Bioavailability contributes to the plasma variance the most (0.34 FOSI) in the dose range 2.5 and 10 mg for 4 h. At 1 h for all doses but placebo, there is a slightly higher sensitivity, but it is reduced at 2 h. From the other parameters, only the impact of the central volume and clearance on the predicted plasma variance are depicted (Fig. 8b and c, respectively). As expected, the influence of V_1 is greatest before distribution equilibrium is reached (1 h), regardless of the dose given. For placebo, the contribution of V_1 is zero. Also, as expected, the contribution of clearance to the variance of the predicted plasma concentration is greatest during the elimination phase, regardless of the dose administered. The contribution of clearance to the plasma variance increased with time for every dose except placebo. The first and total sensitivity indices do not differ significantly, which indicates that there is only a small degree of interaction among the factors. Overall, it can be concluded that for plasma, the naratriptan PK model resembles our understanding of its disposition kinetics in the body.

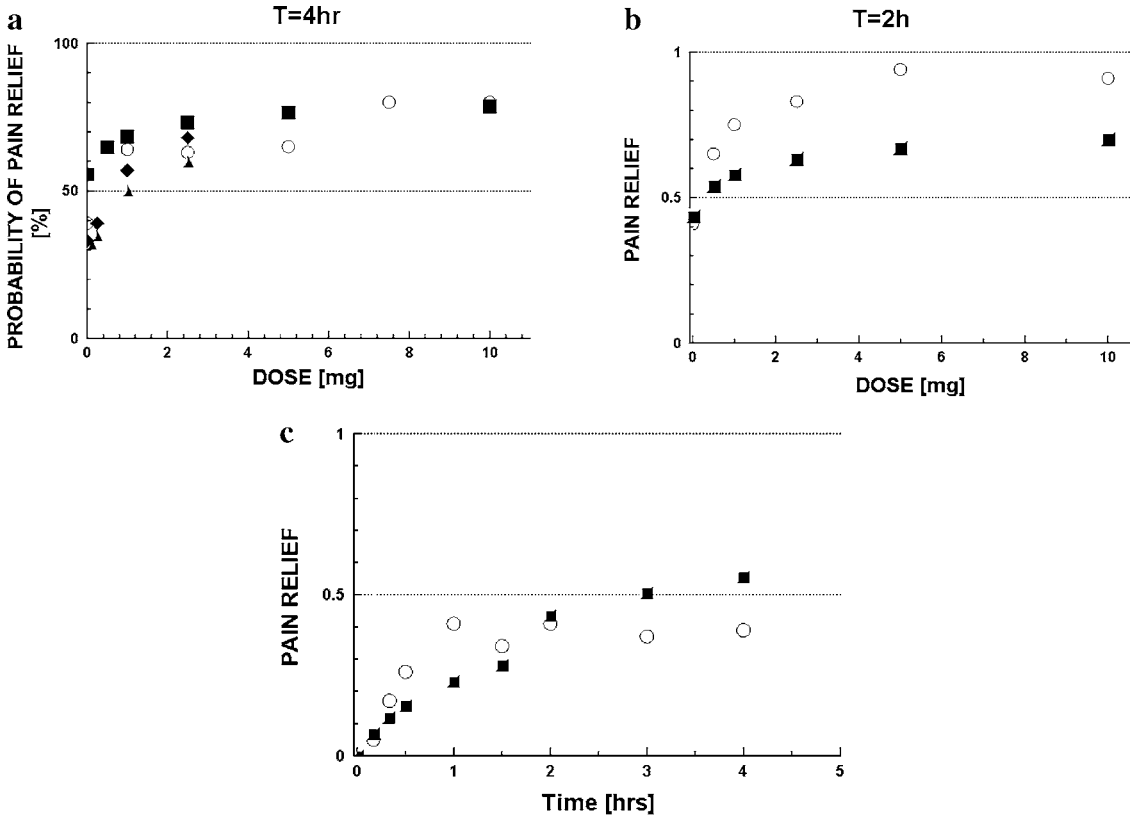

Fig. 7. Predicted probability of pain relief (defuzzified values given as squares) (a) at different doses at 4 h together with observed probability of pain relief from two dose ranging studies reported by Ref. (9) (open circles and black triangles) and one by Ref. (25) (diamonds), (b) at different doses at 2 h together with observed probability of pain relief reported (13) (open circles), and (c) with time associated with placebo administration and observed pain relief reported (13) (open circles).

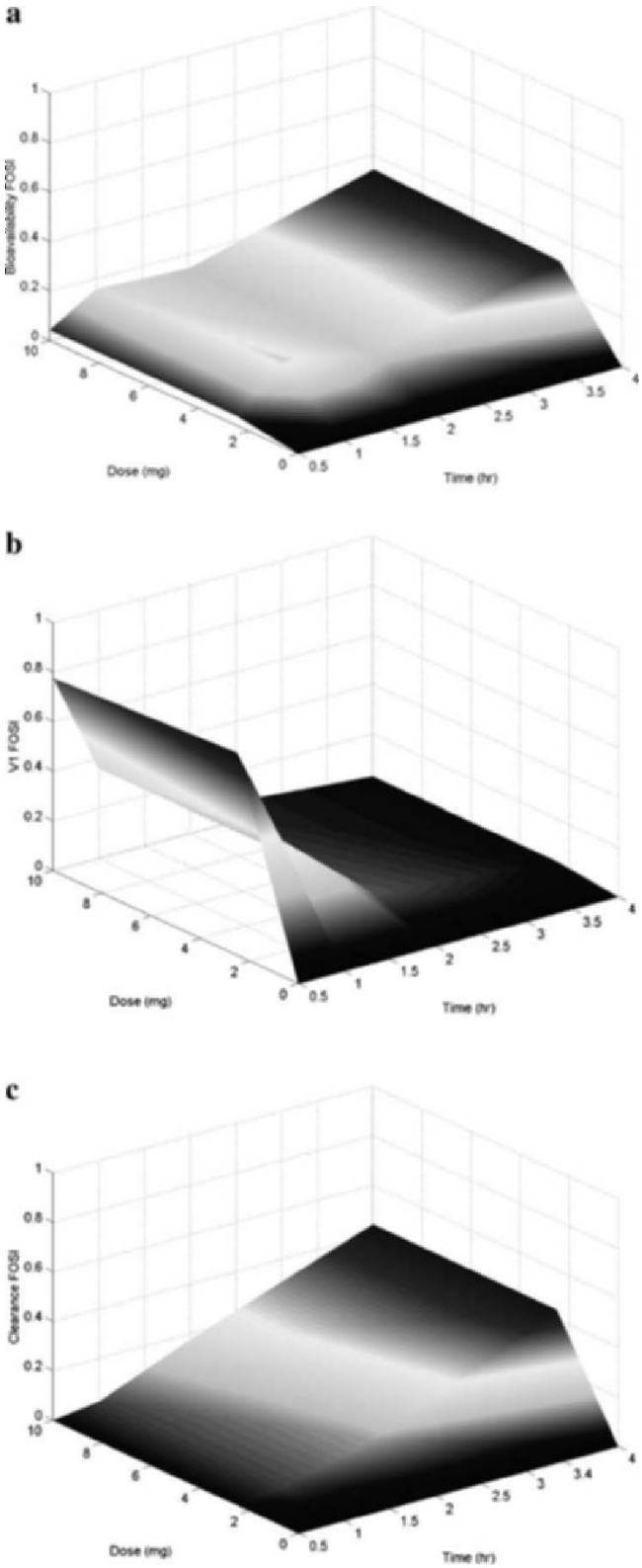

Fig. 8. Sensitivity indices of predicted plasma concentration at different doses with time due to (a) bioavailability, (b) central volume (V_1), and (c) clearance(CL).

The contribution to the variance of the concentration at the effect site (C_{eff}) has also been investigated using the extended FAST method. In this case, the factors are the same as before, i.e., F, ka, CL, V_1, V_2, and CL_d, with the equilibration rate constant (keo) being an additional factor. The effect site concentration variance is similar in its temporal profile to the plasma concentration variance dependencies. The impact of keo is highest (0.29 FOSI) at doses between 2.5 and 10 mg at 1 h (not shown). Beyond 2 h, the impact of keo is negligible for all doses.

The variance of the PD model predicting the probability of pain relief has been also decomposed into the different factors. This proved to be the most interesting analysis, which revealed why the mispredictions noticed in Fig. 7, and also reported by Ref. (6), occurred. There are three factors contributing to the variance of the probability of pain relief—the intersubject variability, the potency factor, and the effect site concentration. The last factor reflects the effect of the PK and link models and can be further decomposed into the contribution of the variances in F, ka, CL, V_1, V_2, CL_d, and keo factors. The impact of the potency

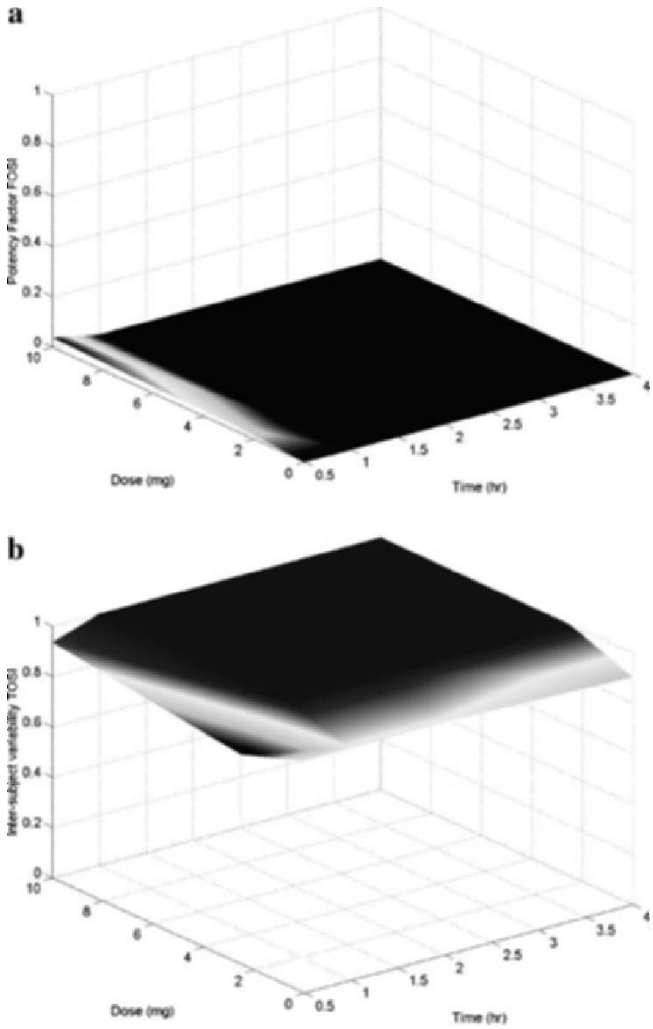

Fig. 9. Sensitivity indices of predicted probability of pain relief at different doses with time due to (a) potency factor and (b) intersubject variability.

factor on the probability of pain relief variance is negligible at all doses for the studied time range (Fig. 9a). Even at its highest, which occurs with 10 mg at 0.5 h, the potency factor sensitivity index is only 0.037 FOSI. The effect site concentration impact to the variance of the predicted probability of pain relief was found to be almost negligible (not shown). The effect site concentration has a very small contribution to the probability of pain relief variance at 0.5 h for all doses except placebo.

The calculated TOSI of the intersubject variability on the variance of the probability of pain relief is shown in Fig. 9b. Between 1 and 4 h at doses ranging from 2.5 to 10 mg, there is no variance in the predicted probability of pain relief, which has reached a maximum: the probability of pain relief is due entirely to the intersubject variability. This is represented by the black region at one TOSI (z-axis) in Fig. 9b. This signifies that the PK and link models used in the analysis do not have any significant impact in these regions. Apart from a TOSI of 0.83 at 0.5 h following a 2.5-mg dose, all TOSI were above 0.9, which shows the great importance of the intersubject variability for the prediction of the probability of pain relief.

DISCUSSION

Clinical trials simulation aims to predict outcomes for various possible clinical trials by integrating and utilizing all the available information and knowledge gained during earlier stages of drug development. The information may often be quite limited, semiquantitative, and uncertain as CTS makes use of data from phase II, I, and even preclinical studies. As such, uncertainty analysis implementing fuzzy simulations may be useful in CTS.

Clinical trials simulation is essentially a Monte Carlo (MC) procedure addressing both the typical behavior and departures from it. As with any MC exercise, CTS is quite heavily dependent on a reliable specification of the pdfs for PK/PD model parameters. The whole prediction may be biased or noninformative should the pdfs be compromised. Uncertain and vague parameters are either fixed to a value, stating conservative or optimistic assumptions, or alternatively, if upper and lower bounds are known, a uniform distribution may be assumed (5,7). The first approach, fixing the parameter values, suffers in that not all possible alternatives are explored, when the assumption may be vital for the predictions. The danger of the second approach, which assumes uniform distributions, is providing a complete quantitative characteristic of a random variable and treating an uncertain parameter as fully defined when it is not, and the resultant predictions may therefore be biased. An alternative approach, suggested in the current study, is to represent the uncertain parameter(s) as fuzzy numbers, which to the best of our knowledge has not yet been considered in pharmacokinetic/pharmacodynamic modeling. This approach allows a great deal of flexibility and incorporates qualitative information and prior knowledge.

Our main aim was to incorporate parametric uncertainty when predicting probability of pain relief following naratriptan administration using a previously reported investigation (6). In their study, as discussed above, for parameters that could not be estimated because of lack of data, either a conservative assumption was made, as for the case of potency factor where a nonformal local sensitivity analysis was performed for tlag, or a Monte Carlo simulation was performed with the uncertain parameters (F, ka, keo) set at several levels each with a coefficient of variation at 0, 30, and 60%. Following the first two situations, the uncertain parameters were fixed, whereas in the third case, a CV of approximately 30% did not severely affect the power of the study. Clearly, the assumption about parameters varying with one and the same coefficient of variation does not reflect our knowledge of these parameters. In the present analysis, all the uncertain parameters were specified as fuzzy numbers, reflecting some prior belief. After performing fuzzy simulations, it was found that the prediction for the probability of pain relief was very similar to that reported in Ref. (6) (not shown). This may be attributed to the fact that either the naratriptan PK/PD model was not sensitive to the uncertain parameters or that applying fuzzy sets theory, in this particular study, does not identify anything which has not already been accounted for by probability theory. Global sensitivity analysis offers a useful means of distinguishing between these two possibilities and has been undertaken in the current studies.

Certain limitations of the fuzzy set approach were noticed in the course of the study. The most obvious criticism is that of subjectivity introduced when specifying membership functions. Another more serious limitation is the lack of sound formalism in transforming between fuzzy sets and pdfs. The same observation was also made in one of our previous investigations (17). Unsuccessful attempts were made to transform the pdfs into fuzzy numbers according to the optimal membership function criterion (26). These attempts failed as an optimal fuzzy number based on pdfs with huge coefficients of variation of the order of 88% could not be found. Because of the lack of a better alternative, a normalization technique was used instead. The transformed fuzzy numbers covered approximately the range given by 2σ around the mean value of the original pdf, which was considered satisfactory. This lack of transformation methods may be attributed to the fact that fuzzy sets theory is a relatively new theory, and as such, there are concepts that still need to evolve.

To acknowledge the inherent variability, a number of researchers use Monte Carlo simulations. Compared to still widely used modeling practices based on "typical" (mean) values only, any approach that incorporates measures of the existing variability and uncertainty into the model makes better use of the prior information available and provides more meaningful results. However, there is a danger when specifying the probability density function for parameters in the presence of qualitative and semiquantitative data, a necessary first step in MC simulation. In many cases, as was seen here, not all model parameters can be estimated. One approach is to fix the uncertain parameters to some value and simulate using the others [as demonstrated in Ref. (6)], but this could compromise the whole exercise as it does not include all the existing knowledge/assumptions of the investigators. Another approach, undertaken here, is to specify the uncertain parameters as fuzzy numbers and perform fuzzy simulations. We believe that an important advantage of the fuzzy simulation method is that it can be applied in the presence of vague, qualitative, and semiqualitative

information. However, it would be wrong to consider Monte Carlo and fuzzy simulations as mutually exclusive techniques. Fuzzy simulations should be applied when Monte Carlo technique may produce large biases in the predictions, i.e., handling uncertain, limited quantitative information.

Sensitivity analysis (SA) is not new to the fields of system theory and statistics (27,28). Sensitivity analysis has also been acknowledged in the analysis of compartmental models (29,30). However, there are a very limited number of SA studies of the commonly used one- and two-compartment models (31) and PK/PD models (32,33). Most of the studies lack formalism and are limited to perturbation of one or two parameters and registering the changes on the output. A few applications with formal local SA exist, but they are exclusively in the physiologically based pharmacokinetic modeling area (34–36). Global sensitivity analysis has not yet been applied to investigate the structural uncertainty of PK/PD models. Pharmacokinetic/pharmacodynamic models are mainly selected, and their parameters' mean and variances were estimated based on providing a best fit, according to some criterion to the experimental data. There are quite often competing models providing similar fits to the data, and in such cases, GSA may help to choose the most appropriate model, not only based on fitting to the data but also on testing model sensitivity to its factors and their variances.

To test the hypothesis that the naratriptan PK/PD model was insensitive to the uncertain parameters, sensitivity analysis, in particular GSA, was performed to study three of the major outputs, i.e., plasma concentration, effect site concentration, and probability of pain relief. It was found that the PK and link models behaved as expected. The impact of the uncertain parameters, *F*, ka, and keo, on the variance of the responses was not large, with the highest being 0.2 FOSI for *F* at 10 mg and 4 h. The misprediction observed in Fig. 7 was found to be due to the pharmacodynamic model, where there was an overwhelming influence of the interindividual variance. The variance of the predicted probability of pain relief is dominated by the variance of the interindividual factor (see Fig. 9b). The contribution of the variance in the PK and link models, reflected by the effect site concentration, to the predicted variance of the probability of pain relief was measurable only up to 0.5 h, as at later times, the intersubject variability dominates the predictions. However, even at that time (0.5 h), the effect site concentration failed to have a noticeable impact on the variance of the predicted probability of pain relief (results not shown).

The entire analysis demonstrated that the uncertain parameters do not have great significance. This is the reason why the predictions from the fuzzy and Monte Carlo simulations were very similar. Both predictions were heavily influenced by the interindividual variability. It may be concluded that if the only aim was to predict the probability of pain relief following oral (tablet) administration of naratriptan, a much simpler dose response, instead of the more complicated PK/PD, model would have yielded very similar, if not identical, predictions. The naratriptan PK/PD model predictiveness may be improved by either refining the PD model or better still by specifying the interindividual error by additional data collecting with improved design, and indeed optimal design (37). Although the predictions made and the conclusions reached in this study are specific to naratriptan, the same methodologies may be applied to other drugs and studies. Uncertainty analysis on both parameters and model structure has to be an integral part of any PK/PD modeling exercise. Moreover, modeling is an iterative process, where the results and/or conclusions from sensitivity and Monte Carlo/fuzzy simulations need to be reanalyzed with the PK/PD model to improve it.

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