PKPD relationships and dose rationale in analgesic drug development : towards the prediction of target engagement

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CHAPTER 6

Semi-mechanistic modelling of the analgesic effect of gabapentin in the formalin-induced rat model of experimental pain

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ABSTRACT

Purpose: The formalin-induced rat model of nociception involves moderate continuous pain. Formalin induced pain results in a typical repetitive flinching behaviour, and these data display a biphasic pattern characterised by peaks of pain We described the time course of pain response and the analgesic effect of gabapentin using a semi mechanistic modelling approach.

Methods: Male Sprague-Dawley rats received gabapentin (10-100 mg/kg) or placebo 1 hour prior to the formalin injection. A reduction in the frequency of the 2nd peak of flinching was used as a behavioural measure of gabapentin mediated anti-nociception. The time course of the flinching response was modelled using a mono-exponential function to characterise the first peak and an indirect response model with a time variant synthesis rate for the second. PKPD modelling was performed using a population approach in NONMEM v.7.1.2.

Results: The time course of the biphasic behavioural pain response was adequately described by the selected model, which included separate expressions for each phase. Gabapentin was found to reversibly decrease, but not suppress the flinching frequency of the second response peak only. Mean IC_{50} values ($\pm$RSE%) were 7510 (40.03) ng/ml.

Conclusions: A compartmental, semi-mechanistic model provides the basis for further understanding of the formalin-induced flinching response and consequently to better characterisation of drug properties, such as potency in individual animals. Despite high exposure levels, model predictions show that gabapentin does not completely suppress behavioural response in the formalin-induced pain model.

ABBREVIATIONS

PKPD= pharmacokinetics and pharmacodynamics, RSE=Relative Standard Error, GABA= gamma amino butyric acid, IIV= inter-individual variability, CV= coefficient of variation, MOFV= minimum objective function value, VPC= visual predictive check, CI= confidence interval, COX-2=cyclooxygenase-2, NMDA= N-methyl d-aspartate, NK1= neuroenkephalin 1, MED= median effective dose.

INTRODUCTION

Ideally, the evaluation of the efficacy of novel treatments for neuropathic pain should be based on pre-clinical models that mimic not only the symptoms of disease, but also consider the substrates underlying the pathophysiology of nociception in humans, i.e., show construct validity [1]. Nevertheless, most behavioural models of pain rely on withdrawal responses to evoked pain, which reflect sensory perception and consequently one's ability to discriminate its intensity, localisation and modality [2, 3]. As such, these measure ignore other features of human pain [4].

Regardless of the potential limitations mentioned above, the formalin induced pain (FIP) model is a well-accepted screening test. The method comprises moderate, continuous pain due to tissue injury following injection of formalin. In the FIP model, the observed behaviour in response to a painful stimulus, assessed as flinching frequency, is used as measure of efficacy [2, 5, 6]. This behavioural measure is thought to reflect both the sensory and emotional aspects of pain [7, 8]. From a mechanistic perspective, the presence of common elements of human pain behaviour in the FIP model makes it possibly one of the most predictive models among the available experimental models of acute pain. These properties have also made the FIP model an appealing tool for the screening of compounds showing potential central anti-nociceptive activity [6, 9]. In fact, various compounds have been found to affect flinching behaviour (e.g., indomethacin and Na^{+} channel blockers), as assessed by the inhibition of the second pain peak, which corresponds to the processes underlying peripheral and central sensitisation [2, 10].

In the current investigation, we evaluate the pharmacokinetic-pharmacodynamic properties of gabapentin in the FIP model. Gabapentin is believed to act via antagonism of voltage gated Ca^{++} channels in afferent neurons, thereby indirectly affecting GABA activity [11]. It has been shown to affect the amplitude of the second pain peak, whilst leaving the other components of the pain response largely unaffected [12].

Despite the widespread use of gabapentin as a reference compound in preclinical models, no quantitative methods have been implemented so far that allow discrimination between drug and biological system properties, and consequently provide a more consistent ranking of candidate molecules. The availability of PKPD relationships would also serve as the basis for translating drug (analgesic) effects across species [1]. The use of PKPD modelling offers an opportunity to better understand the *in vivo* time course of pharmacological effects, providing further insight into the mechanisms of action [13, 14]. Nonetheless, these concepts have been underutilised in pre-clinical pain research [15]. This may be explained, at least partly, by the lack of pharmacokinetic information and the absence of the time course of treatment response [1].

The primary goal of this study was therefore to develop a semi-mechanistic model that allows the characterisation of the time course of formalin-induced pain and assess the effects of gabapentin on flinching behaviour. In addition to known experimental issues such as high variability in response, we show that the main challenges for the characterisation of PKPD relationships using experimental behavioural pain models are the lack of pharmacokinetic information and the absence of the time course of treatment response. Lastly, we explore the relevance of parameter estimates by comparing our findings with published data from other experimental models of pain as well as with clinical data in neuropathic pain patients.

MATERIALS AND METHODS

Experimental Design

Protocols and experimental procedures were reviewed and approved by the Home Office, UK, as required per project licence. The experiments were performed following approval by the Ethics Committee. Sprague-Dawley rats (Charles River, UK weight range 100-300 g) had metal bands attached to their right hind-paws and were placed in Perspex recording chambers and allowed to habituate for 15 min before administration of formalin. The animals were then injected with 50 µl of formalin, subcutaneously in the ventral surface of the right hind-paw at a 2.5% conc/vol. Following formalin administration, animals were returned to the Perspex recording chambers and the number of flinches was counted by the automatic teller for 1 hour. Four rats could be tested in parallel using this system. All animals were euthanized at the end of the experiment.

Gabapentin or vehicle was administered orally at doses of 0, 10, 30, 100 mg/kg approximately 1 hour prior to formalin administration. In 4 of the experiments, the animals were randomised to either the placebo of the 100 mg/kg dose group, while in the 5th there were 2 additional dose groups who received 10 or 30 mg/kg respectively. In each experiment,8 animals were allocated to a particular dose level. Data from five different experiments were pooled together, making a total of 96 animals.

Pharmacodynamic measurements

The total frequency of flinches was recorded at 5-min intervals, from 5 to 60 min after administration of formalin.

Data Analysis

Pharmacokinetic simulations

Gabapentin concentrations were simulated using a previously published model based on two-compartment drug disposition and dose-limited absorption (16). The model was built in a stepwise manner. First, intravenous data from a previous experiment was modelled to obtain disposition parameter estimates, namely clearance and volume of distribution. Subsequently, absorption parameters (bioavailability and input rate) estimates were obtained for oral data. More information on these experiments can be found in the appendix. This pharmacokinetic model is described by the following expression [16]

$$C = \frac{k_a FD}{V_1}\left\{\frac{(k_{21-}\lambda_1)e^{-\lambda_1 t}}{(k_a-\lambda_1)(\lambda_2-\lambda_1)}+\frac{(k_{21-}\lambda_2)e^{-\lambda_2 t}}{(k_a-\lambda_2)(\lambda_1-\lambda_2)}+\left(\frac{(k_{21-}K_a)e^{-K_a t}}{(\lambda_1-k_a)(\lambda_2-k_a)}\right\}\right. \quad (1)$$

where $k_{a=}$absorption rate constant, V_1=central volume of distribution , F=bioavailability of the administered dose, λ_1 and λ_2 correspond to the initial and terminal slopes representing bi-exponential decline respectively and k_{21} is a rate transfer microconstant between compartments 1 and 2. A summary of the PK model parameters is shown in Table 6.1.

An analytic solution to the 2 compartment model, implemented in NONMEM was used for the simulation and the derivation of the above expression from the estimated primary parameters (Volume, Clearance) and is elaborated in the appendix.

Exploratory data analysis

Before starting model building, we performed a graphical evaluation of trends in the experimental data, including the time course of gabapentin in plasma, the effect vs. time and the concentration vs. effect relationships. To ensure suitable model parameterisation and assess the existence of correlations in the data, pain response at any given point in time was also plotted against the preceding interval. Such correlations are of relevance for modelling purposes, as highly correlated data may lead to model misspecification. In fact, pain response (flinching frequency) at a given sampling time has been shown to correlate with preceding measurements [17, 18].Given that the frequency of flinches /time interval was high>10, we decided to model the counts as continuous data.

Table 6.1: Pharmacokinetic parameter estimates used for deriving simulated concentrations at the time of measurement of the response.

Parameter	Values
Pharmacokinetic	
Central compartment volume (V_1)	0.118 (l)
Peripheral volume (V_2)	0.253 (l)
Clearance (Cl)	0.159 (l/h^{-1})
Intercompartmental clearance(Q)	1.22 (l/h^{-1})
Bioavailability fractions(F)	1,0.75,0.22[a]
Absorption constant (Ka)	0.26 (h^{-1})

[a] *For doses 10, 30,100mg/kg respectively*

PKPD model parameterisation

In the FIP model, there is a temporal delay between the appearance of gabapentin concentrations in plasma and the onset of pain response. Depending on the half-life of the compound, the analgesic is administered before the induction of hyperalgesia with formalin. Given that two pain peaks consistently occur after administration of formalin, this phenomenon was parameterised in terms of two independent pharmacodynamic (PD) compartments. The first peak (i.e., pain associated with the first phase) was described by the following exponential decay relationship:

$$\frac{dFO}{dt} = -K_{df} * FO$$
$$PAIN_1 = F_3 * FO \qquad (2)$$

where *FO*= formalin-induced stimulus, k_{df}= dissipation constant for formalin, F_3=basal pain load in the first PD compartment, $PAIN_1$= total pain in the first PD compartment

The first peak of pain is almost instantaneous following algogen administration, thus the parameter F_3 reflects pain at baseline, which wanes spontaneously soon thereafter.

The onset of the second peak of pain is after a quiescent phase and is considered to reflect the central hypersensitisation which ultimately manifests itself as a 2nd more prolonged phase of flinching. Similarly to the first peak, pain intensity increases to a maximum and then remits spontaneously. Given the lack of a direct correlation between the gabapentin concentrations in plasma and effect over time, an indirect model was deemed to be most appropriate to describe this 'turnover' of the pain response [19].

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In these models the measured response (*R*) is assumed to result from factors controlling either the input or the dissipation of the response. The general expression to describe these models is given by the expression below:

$$\frac{dR}{dt} = k_{syn} - k_{deg} * R \tag{3}$$

where *dR/dt* is the rate of change in the response over time. k_{syn} represents the zero-order rate constant for the formation of the response and k_{deg} the first-order rate constant for loss of the response. We have replaced the response *R* in equation 3 with the term *FL* to make explicit reference to the time course of the flinching response triggered by the central sensitisation in the spinal cord following the first peak.

$$\frac{dFL}{dt} = k_{syn} - k_{deg} * FL \tag{4}$$

Given that the pain response wanes with time i.e., there is spontaneous recovery within 1 hour after injection of the algogen [2, 6], k_{syn} was treated as time-dependent variable.
Depending on whether *t,* the time after formalin injection, was larger or smaller than *Tlag* (i.e., the delay between the occurrence of the first and second peaks of pain), different estimates were considered for k_{syn}. Thus for $t \geq Tlag$, model parameterisation described the onset of the second phase of pain. If $t<Tlag$, $k_{syn}=0$, which meant the second phase of pain had not yet begun. A modified gamma function was required to describe the time course of k_{syn} and equation 4 was thus transformed to an expression representing the natural change in pain frequency , described by the following expression:

$$K_{syn} = A * (\alpha^{(t-lag.t)}) * e^{(-\beta(t-lag.t))} \tag{5}$$

Where *A* (response unit h^{-2}), α (a dimensionless constant), ß (h^{-1}) are the parameters of the gamma function describing the time course and intensity of the second phase of pain as assessed by the frequency of flinching.
As mentioned earlier, the time course of the disease is a result of the temporal change in the frequency of flinching represented by *FL*. At the start of the study, i.e., before onset of the 2nd peak, the frequency of flinching was assumed to be 0. Consequently, the generic equation 4 can finally be rewritten in terms of *FL* as follows:

$$\frac{dFL}{dt} = A * (\alpha^{(t-lag.t)}) * e^{(-\beta(t-lag.t))} - K_{deg} * FL \tag{6}$$

The model was applied to simultaneously fit both placebo and gabapentin data. It has been observed that drug response further decreases the frequency of flinches and thus

it is superimposed on the natural disease process. Gabapentin effects *(DEFF)* were best described by an inhibitory I_{max} function, which represents the reversible counteracting effects of gabapentin on the algogenic action of *FL,* i.e., the observed flinching behaviour:

$$DEFF = (1 - \frac{I_{max} * C_p}{IC_{50} + C_p}) \quad (7)$$

where I_{max}= maximum possible inhibition of pain, C_p=plasma concentration and IC_{50}=plasma concentration at which 50% of the inhibition occurs. As gabapentin only affects the second peak of pain, we assume gabapentin effects reflect a decrease in central sensitisation. It should be noted that indirect response models incorporate the Hill function directly in the turnover differential equation whereas we have chosen to parameterise gabapentin effect (*DEFF*) directly on the pain variable of the 2nd peak, rather than within the differential equation. This is because the analgesic does not alter the onset of the pain nor its eventual disappearance, but reversibly alters its peak intensity. In other words, the analgesic effect of gabapentin is a covariate on the behaviour or flinching response. A similar approach has been used previously to describe the effects of lumiracoxib on COX-2 inhibition [20].
The net pain observed is the product of the gabapentin effect *(DEFF)* and *FL* or the resulting PD compartment *(PAIN2)*

$$PAIN2 = (1 - \frac{I_{max} * C_p}{IC_{50} + C_p}) * FL \quad (8)$$

The total pain (*PAIN*) was described by the sum of the pain in the two model compartments:

$$PAIN=PAIN1+PAIN2 \quad (9)$$

A schematic representation of this mechanistic PD model is presented in Figure 6.1**.**

Interindividual variability was modelled exponentially and applied serially to each parameter. Stochastic parameters were retained in all cases which showed significant improvements in the model, as defined by statistical criteria described below. Residual variability was best described by an additive error model.

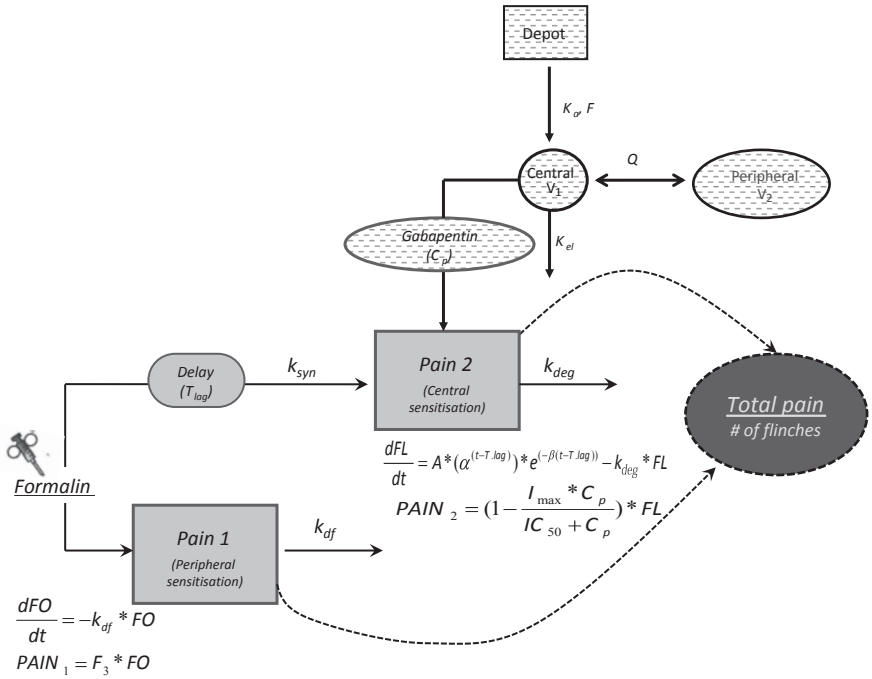

Figure 6.1: Schematic diagram of the pharmacokinetic-pharmacodynamic model. PK compartments are displayed with dashed horizontal (blue) hatching, while PD compartments' are dark shaded (grey or black).

Model diagnostics and validation

Model selection was based on the visual examination of the goodness-of-fit plots using Xpose version 4.2.1[21], the precision of model parameter estimates is represented by the coefficient of variation [(%), computed as the ratio between the standard error provided by NONMEM and the parameter estimate multiplied by 100, and the MOFV provided by NONMEM. The difference in the MOFV between two hierarchical models was considered statistically significant if the MOFV changed by 6.63 points which is equivalent to a p value of <0.01 for a χ^2 distribution. The final model was further evaluated based on visual and numerical predictive checks and bootstrap procedures [22, 23]. Using the final model, the 2.5th, 50th, and 97.5th percentiles from simulated pain response (n=500) were calculated and compared to the experimental data.

NONMEM 7.1.2 was used in conjunction with PsN 3.2.12 for all estimation and simulation procedures. Modelling was based on the first-order conditional estimation method with the INTERACTION option [24]. R statistical software (v 2.10) was used for data manipulation, statistical and graphical summaries [25].

Bootstrap

A nonparametric bootstrap with re-sampling was performed to estimate the confidence intervals for the parameters [23]. This technique consisted of repeatedly fitting the model to replicates of the data set using the bootstrap option in PsN 3.2.12. Parameter estimates for each of the replicate data sets were obtained. The results of successful runs from 500 bootstraps were obtained, and the median and 2.5th and 97.5th percentiles (denoting the 95% confidence interval) determined for estimated parameters.

RESULTS

Data analysis

Pharmacokinetic simulations

Gabapentin concentrations were obtained by simulation at each of the sampling times used for the pharmacodynamic measurements. A two-compartment model with first-order absorption obtained in a previous analysis was used for the purpose of this study. The PK model parameters are summarised in Table 6.1.

Exploratory Analysis

The typical concentration time profiles are shown in Figure 6.2 **(**left panel) with the corresponding time courses of the flinching frequency for the tested doses (right panel). It can be appreciated here that gabapentin only reduces the amplitude of the 2nd peak in a dose-dependent manner.

The disconnect between the two time courses are shown in the figure below with respect to the initiation of the experiment (formalin injection) and the PD observation window. From these it is clear that during the observation window, while the pain response begins and ends the drug is still in the distribution phase. Considerable variability in the response is also evident. From these plots, it is clear that during the experimental protocol, the pain response begins and ends while gabapentin is still predominantly in the absorption phase. Considerable variability in the response can also be seen between animals.

In Figure 6.4**,** the flinching frequency is depicted against time and gabapentin concentrations, stratified by dose level. From the two panels it can be seen that the concentration-effect relationship can be superimposed on the time course of response itself. The data suggests that gabapentin effects have limited effect on the time course of the second pain peak. Furthermore, this phenomenon is further confounded by high degree of correlation between consecutive measurements. Details are shown in the supplemental material (appendix, Figure 6.7).

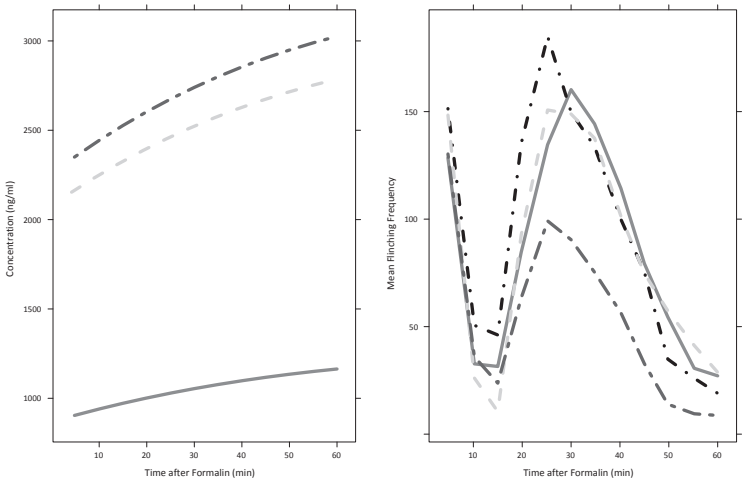

Figure 6.2: Population curves for simulated gabapentin concentrations in the plasma for doses 10, 30, 100 mg/kg (left panel) and the observed flinching behaviour in the formalin-induced model following placebo (dot-dashed) ,10 (solid), 30 (dashed) and 100(dash-dash) mg/kg curves (right panel).

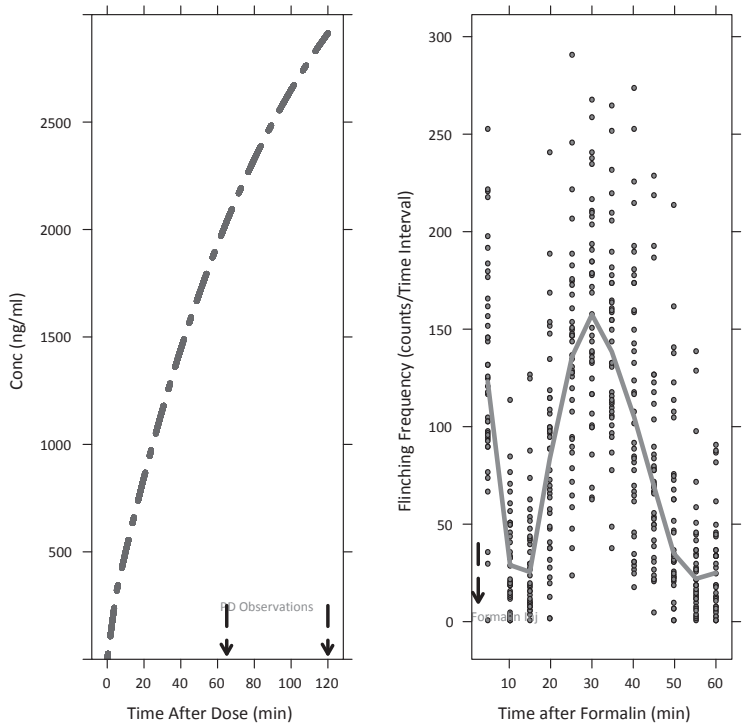

Figure 6.3: Disconnect between the (observed) onset of response (right panel) and the (simulated) time course of concentrations in plasma following a typical dose of 100 mg/kg gabapentin (left panel). Dots represent the individual observed flinching response; the solid line depicts the median response profile.

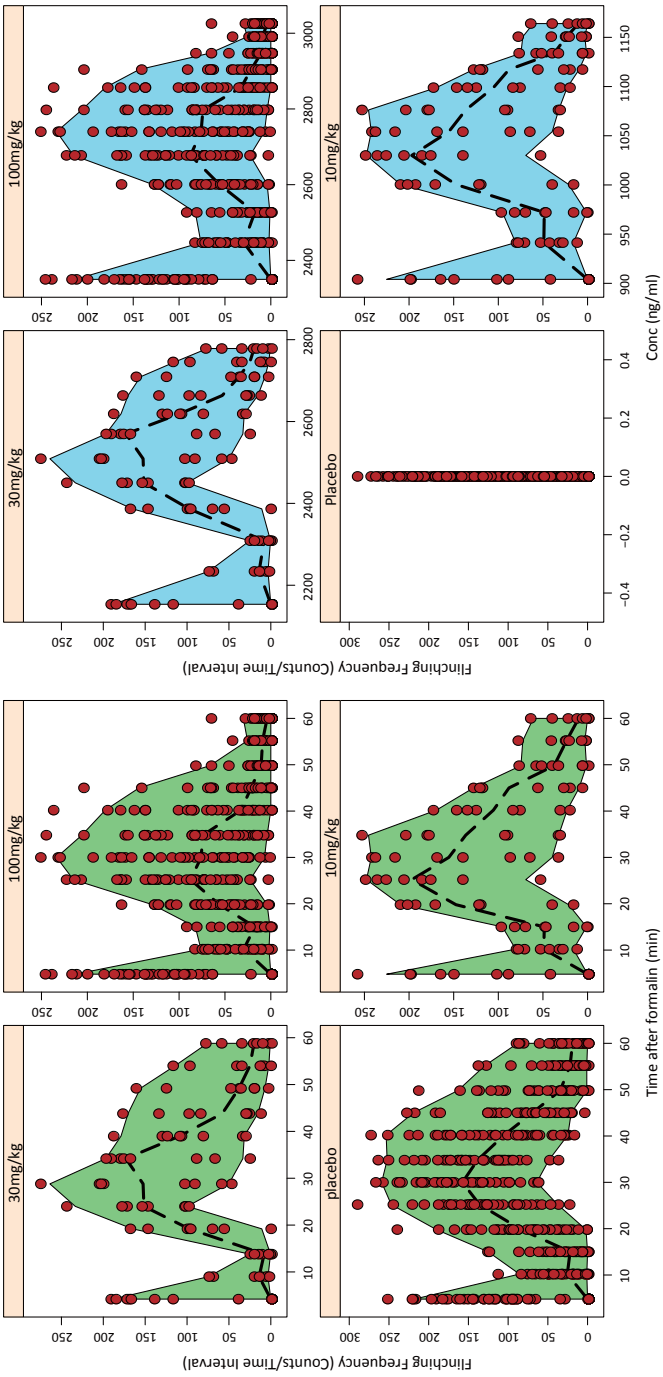

Figure 6.4 Flinching frequency *versus* time (left panel) and gabapentin concentrations (right panel), stratified by dose level.

PKPD modelling

The time course of the flinching behaviour as well as the inhibitory effects of gabapentin following drug administration were accurately characterised by the chosen indirect response model. The structural model described all three components of the pain response to formalin. The goodness of fit plots are presented in Figure 6.5. Sample individual fits are depicted in Figure 6.8 of the appendix. All structural model parameters were identifiable for the current dataset as evidenced from the RSEs (<40%) shown in Table 6.2 below.

Table 6.2: Parameter estimates from the final population PKPD model, including bootstrap estimates and confidence intervals.

	Final PD Model			Bootstrap Estimates			
Parameter	Estimate (CV%)	IIV(CV%)	Shrinkage	Median of Estimate	5 % 95 % Confidence Interval of Estimate	IIV	IIV- 5 % 95 % Confidence Interval
Degradation constant for formalin *(k_{df} h^{-1})*	12.3(5.39)			249.30	12.33,1169.25		
Basal pain load in 1st PD compartment *(F_3 # counts)*	126(4.38)	57.44(21.83)	16.70	132.04	122.32,139.63	0.155	0.1,34.64
*Delay between 1st and 2nd peaks *(T.lag h^{-1})*	0.29(0.20)			0.242	0.0,0.30		
**parameter of gamma function *(A h^{-1})*	2720(6.65)	66.33(17)	14.08	2275.85	1099.145,2943.12	39.03	27.56,46.80
Dimensionless gamma function parameter*(alpha)*	2.29(239)			24.84	1.33,159.012		
parameter of gamma function *(beta h^{-1})*	8.37(30.82)	67.08(78)	15	9.29	5.43,12.17	25	7.21,44.84
Degradation constant for waning of Pain 2*(K_{deg} h^{-1})*	5.97(4.22)			5.347	2.90,6.38		
(EC_{50} ng ml^{-1})	7510(40.33)			6380.5	3961,15390		
Residual Error(additive)		29.09(7.8)	9.40	35.34	33.07,43.77		

IIV is presented as a percentage

* *T.lag is relative to the time after formalin injection*

** *While A, beta are time-dependent parameters, alpha is a dimensionless constant*

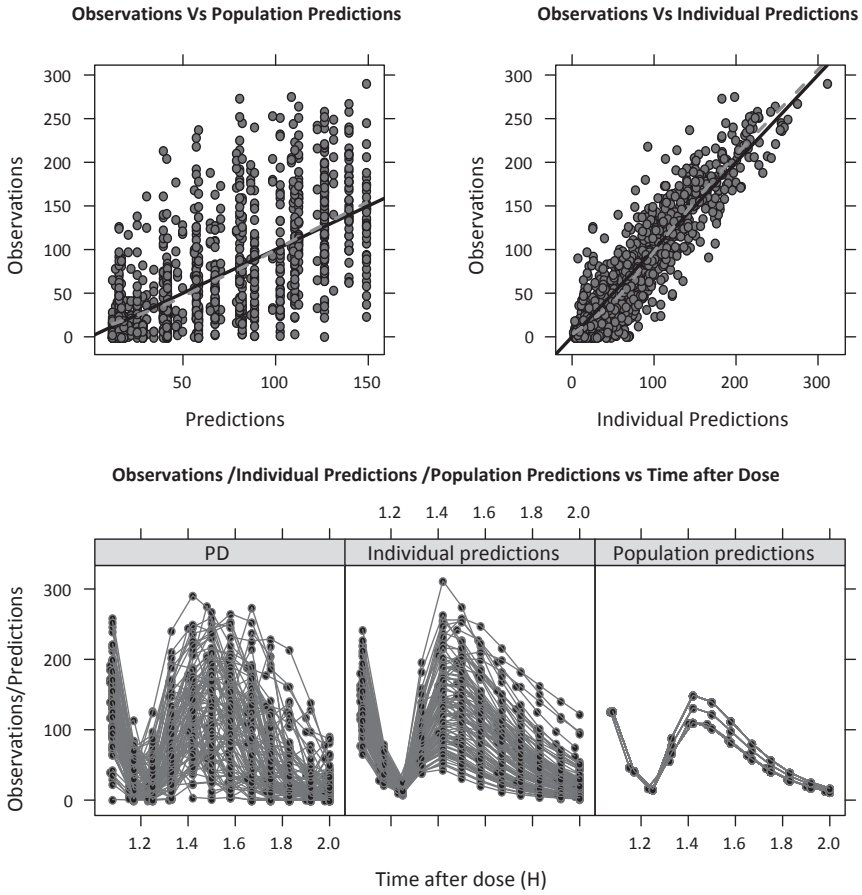

Figure 6.5: Goodness of fit Plots. The upper panels show the correlation between observed and population (left) or individual (right) predicted response. In the lower panels, the observed and predicted responses are depicted over time.

We have assumed the I_{max} to be 1, i.e., the maximum possible inhibition of pain. In practice, however, this is not the case, as the hypersensitisation attains different peak intensities in different subjects [18].

As can be seen from Figure 6.3 and Figure 6.4, there was considerable variability in the data. IIV was modelled exponentially and tested serially on all model parameters. The data supported the inclusion of IIV on the F_3 parameter of the first peak, ß, and k_{deg} on the second peak, resulting in significant drops in the objective function value i.e., yielding statistically significant improvements in the model ($p<0.01$).

Model validation

Model VPCs stratified by dose level are shown in Figure 6.6. The model is able to describe both the median trends in the data as well as the distribution i.e., the interquartile ranges. Since there was more data available for the placebo and 100mg/kg dose, the predictions for these dose levels are comparatively better than for the remaining dose levels. Approximately, less than 5% of the observations fall outside the prediction intervals. The model predicted 2nd peak response occurs slightly earlier than that of the real data.

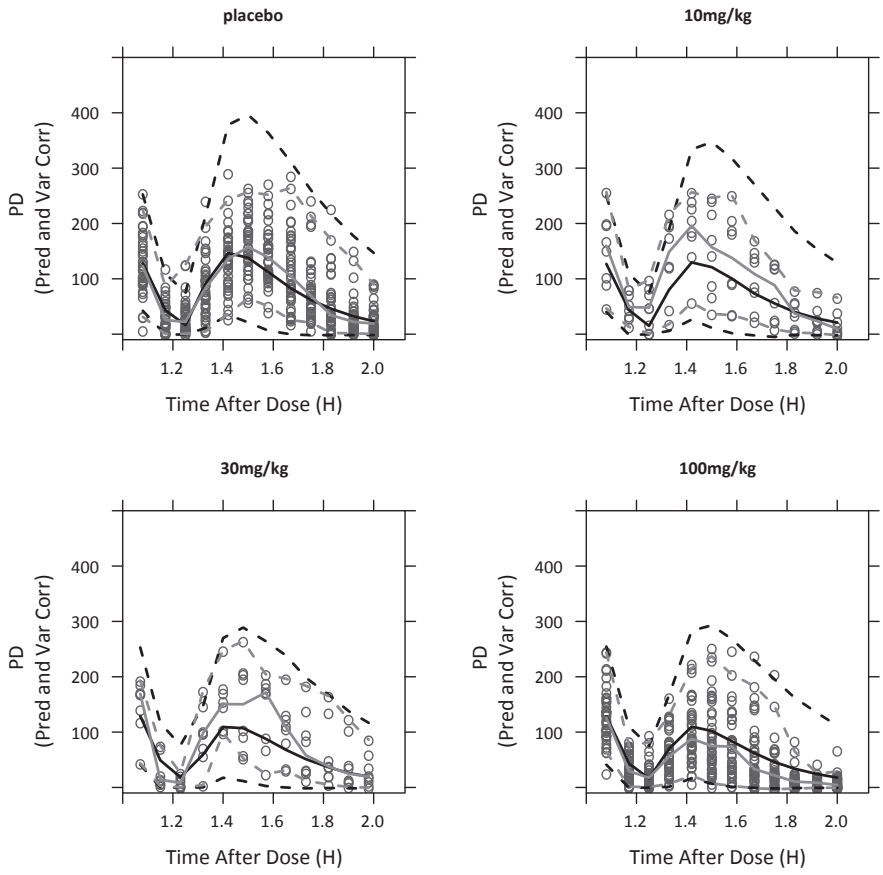

Figure 6.6: Visual Predictive Check for the final PKPD model stratified by dose. The results are based on 500 replicates. Open Circles are the raw data; the red and black lines denote the median of the observed and simulated data while the corresponding dashed lines represent the 2.5th and 97.5th percentile of the observed and simulated data respectively.

Table 6.3: Numeric Predictive Checks- Comparison of median of Observations at the maximum and minimum with corresponding simulations with 95% Prediction Intervals for Pain Phase 1 & 2 by Dose.

	Peak 1, Max PD (5min after formalin)		Peak 1, Trough PD (10min after formalin)		Peak 2, Max PD (30min after formalin)		Peak 2, Trough PD (60min after formalin)	
Dose	Median Real data	Median Simln data (95% CI)	Median Real data	Median Simln data (95% CI)	Median Real data	Median Simln data (95% CI)	Median Real data	Median Simln data (95% CI)
0mg/kg	123.12	128.09 (109.49-148.49)	22.14	16.48 (5.65-27.80)	157.43	137.88 (107.44-173.76)	19.09	23.46 (9.90-38.71)
10mg/kg	159.62	126.90 (90.41-170)	48	14.90 (0.30-39.60)	157.03	121.03 (71.33-192.02)	9.73	21.62 (1.05-51.41)
30mg/kg	169.13	130 (91.60-174.90)	8.17	18.50 (0.43-43.20)	171.52	87.92 (44.11-143)	19.70	19.64 (0.47-49.40)
100mg/kg	126.51	128.51 (110.60-150)	17.50	16.20 (5.40-27.84)	75.01	102.03 (80.02-127.32)	4.40	17.75 (5.12-32.35)

Numerical predictive checks are depicted in Table 6.3, where the median number of flinching counts for observed and simulated (95% CI) data is shown at four different points, with the objective of characterising the maximum and minimum values of the two pain phases. In general, predictions for the placebo and 100mg/kg doses are better as compared to the other two, except in the case of the trough response for peak 2 where the model seems to over predict the frequency of counts while under predicting gabapentin effects, for the top dose as compared to the other dose levels.

Bootstrap

Table 6.3 shows the results of final model parameter estimates along with the results from the bootstrap for 500 runs (median, 5 and 95% CI). Most model parameters were well estimated, with the exception of k_{df}, α, and *Tlag* as can be seen from the wide confidence intervals in the bootstrap. This suggests that not all parameters may be identifiable in subsets of the original dataset. However a sensitivity analysis showed the variation in these parameters did not have significant effects on the overall model fit. Similarly, the parameters describing IIV were not well estimated in the bootstrap. We experienced a high minimisation failure rate in the bootstrap (~70%), which has caused a possible underestimation of IIV during bootstrapping. Therefore, all final IIV estimates are based on objective function criteria used during the initial fitting procedures.

DISCUSSION

Despite its wide use in the screening of compounds for neuropathic pain, till recently no attempts had been made to characterise PKPD relationships in the FIP model, with the exception of the recent work of Velez de Mendizabal *et al.* on lumiracoxib [20]. Though the subject of both investigations was the same (the FIP model) the applications were different. We are interested in describing exposure response relationships of gabapentin while they have studied lumiracoxib topical versus intrathecal drug interactions.

Consequently, quantitative approaches in either case were different. Thus while we have explored exposure~response relationships, Velez de Mendizabal et al have used a KPD (kinetic pharmaco-dynamic) model. In contrast to previous investigations using the FIP model, the semi-mechanistic approach proposed here fulfils two important conceptual requirements for the purposes of compound screening, i.e., it describes the time course of the disease and the gabapentin effect in an independent manner. In addition, our choice of parameterisation took into account the possibility and importance of generating evidence of PKPD properties that can be easily used to translate treatment effects across species. Therefore, model parameterisation has not relied on typical measures such as cumulative response, which despite being technically less demanding has important drawbacks. For instance, if data were to be modelled using cumulative flinching counts, gabapentin potency

would be expressed in terms of the time required to halve the maximum response. Such a parameter would have little physiological meaning even though many consider it suitable for ranking of compounds. Moreover, the use of such cumulative measures of response would not warrant a unique PKPD relationship (see Figure 6.4). It became clear during our exploratory analysis that the flinching behaviour induced by formalin produces a unique fingerprint which prevails over any attempt to characterise the underlying exposure-response relationship using direct response models [26].

By contrast, our approach explores drug(compound) and disease properties using independent parameters. From a pathophysiological perspective, the first peak is caused by peripheral sensitisation, whilst the second may reflect central hypersensitisation. The same phenomenon appears to be reproduced in other species such as mice, gerbils, cats, monkeys [6], suggesting the opportunity for wide use of the concepts presented here. Gabapentin effect was therefore parameterised in terms of an indirect response model, which describes the changes in flinching behaviour in terms of a declining exponential expression. The formation rate of such response (k_{syn}) was further characterised by a gamma function, which indicates the time varying course of formalin-induced symptoms, and consequently modifying the classical indirect response model of Dayaneka *et al.* [19]. This function has been previously described for endpoints where spontaneous recovery from inflammation can be expected [14]. Historically, negative power functions of time have been applied to describe clearance curves in PK studies and tracer kinetics in general, with a view to replacing multicompartment analysis. Though non-physiological, they require considerably fewer parameters and yielded more accurate predictions[27]. We have modified the traditional gamma function by parameterising the variable T_{lag} as the exponent of the dimensionless constant α. This led to better fits and lesser numerical difficulties with the minimisation routine. The time to onset of the 2nd peak was about 20 min in our analysis which is in agreement with the observed data and also literature estimates of 10-20 min post formalin[2]. However, there was considerable variability in this parameter as can be seen from the median effect vs. time curves in Figure 6.2. It should also be noted that the I_{max} was defined as the maximal change in pain intensity i.e., return to a baseline state, and therefore set to a theoretical maximum of 1. Fixing of the parameter to a single maximum value was applied even though the disease process and treatment response was not expected to be same in all subjects. The approach has been previously applied by Maas *et al.* to describe migraine pain [18].

Indirect response models incorporate the Hill function directly in the turnover differential equation whereas we parameterise the drug effect (*DEFF*) directly on the pain variable of the 2nd peak and not within the differential equation. This is because the drug does not alter the onset of the pain nor its eventual disappearance but reversibly alters its peak intensity. In other words, the drug effect is a covariate on the behaviour or flinching response. Therefore application of the drug effect to either k_{in} or k_{out} would have been non-mechanistic. This

effect can be visualised in Figure 6.2 where the typical profile for the 100 mg/kg dose exhibits a smaller 2nd peak compared to the placebo time course. Velez de Mendizabal *et al* employed a similar approach to describe the effects of a COX_2 inhibitor lumiracoxib on COX_2 [20]

Focus should also be given to the observed high between-subject variability in the FIP model, a phenomenon that is well known in clinical pain conditions [28]. Although most investigations consider such variability a purely stochastic process which cannot be assigned to any specific source or mechanism, we have tried to estimate between-subject variability for all relevant model parameters, such as I_{max} or IC_{50}. Unfortunately, this was not always supported by the data. Yet, it is reasonable to assume that individual differences in gabapentin potency do exist and occur due to the time varying effects of formalin, which can affect both maximum frequency of flinching behaviour as well as modulate gabapentin effects effects on central hypersensitisation. On the other hand, IIV could be identified for parameters associated with the induction of formalin-induced pain. The basal load of pain (F_3) differed among subjects and an η on this parameter improved the fit. The waning of the pain phenomenon (k_{deg}) was also found to differ among individuals and fitting showed significant improvements when IIV was applied.

Diagnostic and validation plots, such as the VPCs show the model has adequate predictive properties. Ideally, in such circumstances, the next step would be to fit the model to external datasets. Regretfully, we have not been able to identify such data.

Limitations

A potential drawback in our approach is that the IC_{50} estimates appear to be beyond the range of observed gabapentin concentrations. This situation is caused by the use of a theoretical maximum (I_{max}), which was not reached by gabapentin. Had this been the case, the second peak would have been suppressed completely. However, all concentrations tested were in the linear part of the curve. On the other hand, it is well documented that gabapentin produces partial symptomatic relief in neuropathic pain, rather than showing any disease modifying effects. It is therefore plausible to infer that incomplete suppression of the second peak reflects actual clinical effects of gabapentin [29, 30]. Yet, we consider the ability to discriminate between compounds that cause total pain suppression and partial relief highly desirable and do not anticipate any bias in the way compounds can be ranked on the basis of their potencies.

In a situation where $C_p << IC_{50}$, the DEFF in equation 7 would reduce to the following expression:

$$DEFF = (1 - \frac{1}{IC_{50}})$$

It could be argued that the IC_{50} would then be a linear coefficient rather than a true measure of potency and consequently the estimate of the IC_{50} would not be robust.
It is important to mention that we assume that an ideal or an efficacious drug would completely suppress flinching and by fixing I_{max} we assume maximal response is possible in this biological system. The IC_{50} then becomes a relative parameter, conditioned on an I_{max} of 1. If this is the case then this parameter can be reasonably used to compare potencies across compounds. Modelled parameters were not always identifiable in the bootstrap as can be seen in Table 6.2. This suggests that a rich dataset may be required to fit this model and here is where the advantages of a model based approach come in. Existing data may be used in combination with future data whereby the new data are used only to estimate compound specific parameters and existing data support estimation of system specific parameters.
We also acknowledge that the gamma function may have little physiologic basis, and thus future improvements to the model could be aimed at replacing this function with a more physiologic alternative. Such an alternative parameterisation may however require the availability of rich datasets. We anticipate that historical data may be used in combination with newly generated experimental data whereby only analgesic-specific parameters need to be estimated.
Complex pathophysiological processes underlie the generation of second peak, such as the release of various excitatory neurotransmitters acting through NMDA and NK1 receptors which then initiate a cascade leading to central sensitisation [31]. We have parameterized these underlying processes collectively as *FL*, under the assumption that differences in the individual time course of neurotransmitters was not statistically different. This choice was made to ensure description of the observed phenomenon rather than the pathophysiology of the pain response.
We also highlight a few shortcomings of the experimental design in our study, which was performed according to standard experimental procedures. The time of dosing of gabapentin should have been planned taking into consideration potential differences in pharmacokinetic properties. If gabapentin had been administered earlier, the return to baseline of the flinching events might have coincided with it's elimination phase. Secondly, no baseline behaviour was recorded i.e., flinching counts between the administration of gabapentin and that of formalin (T=0). As explained previously, pain burden at baseline also showed differences between animals (η on F_3).

Comparison with other pre-clinical and clinical findings

We have attempted to compare our results with other published pre-clinical and clinical data on gabapentin. Table 6.4 gives an overview of the EC_{50} and ED_{50} values reported for different pain models. Except for one pre-clinical experiment[32] and one clinical study [33] no other publications have applied modelling to analyse or interpret the data. Most authors used

ED_{50} and MED as measures of potency with no mention of concentrations, rendering direct comparisons rather difficult, if not impossible [12, 34-37]. Noteworthy is the wide variability observed in the findings of different authors. There were other important differences such as the ceiling effect being observed by Iyengar *et al.* at a relatively low dose of 50 mg/kg while others reported peak effects between 100-300 mg/kg[31, 37].

Among those studies where direct comparison with our work was possible, Todorovic reported an EC_{50} of 467 nM as compared to 43 nM reported here. More consistent results for clinical EC_{50}s were reported by Lockwood *et al.* (31.28 nM), whilst Whiteside *et al*. provide estimates for clinical MED values of 69.72 nM [38, 39]. Notably, Whiteside's work is the only effort at inter-species correlations, amongst the publications we reviewed, albeit not based on modelling concepts.

CONCLUSIONS

In summary, differences in analgesic potency exist in pre-clinical models, which cannot be interpreted simply in terms of precision. A comprehensive evaluation is missing of the differences and similarities in the underlying mechanisms affected by evoked pain in the various models currently available for pre-clinical evaluation of neuropathic pain.

Clearly, the challenges for the identification of suitable compounds for the treatment of neuropathic pain will not be overcome until adequate biomarkers of pharmacology are identified [40, 41]. Yet, irrespective of such differences in pathophysiology, approaches are required that facilitate the translation of pre-clinical findings and provide the basis for the characterisation of analgesic-specific properties. A parametric, model-based approach is essential to ensure distinction between disease processes and analgesic effects.

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Table 6.4: Comparison of Experimental findings for gabapentin in various published preclinical and clinical studies.

No.	Author	Experimental Model	Study Protocol	Main findings	Comparison with our work/ other remarks
			Comparison with Preclinical experiments		
1.	Shannon (Shannon *et al*., 2005)	FIP in rats	Comparison of anticonvulsants with different mechanisms of action in the formalin test of persistent pain (rats, mice). Gabapentin doses tested were 30-300mg/kg (IP)	In rats efficacy was seen across the dose range from 30-300mg/kg Minimal effective dose (MED) in rats was 30mg/kg for the 2nd peak of pain. The MED for locomotor activity in mice was 100mg/kg	Drug effects on the 2nd peak between 30-100mg/kg
2.	Iyengar (Iyengar *et al*., 2004)	FIP in rats	A comparison of the effects of analgesic agents such as uptake inhibitors, tricyclic antidepressants, anticonvulsants on attenuation of formalin induced late phase paw-licking behaviour. Gabapentin was administered in doses of 10/30/50mg/kg IP	Gabapentin attenuated paw licking behaviour in the doses administered. A plateauing of effects was observed beyond 50mg/kg	We observed analgesic effects at 100mg/kg as well
3.	Hama *et al* (Hama and Sagen, 2007)	Rat model of acute NP resulting from experimental spinal compression injury	A placebo controlled 12 week study. A number of compounds among which opioid analgesics, antidepressants, anticonvulsants were tested. Gabapentin was administered at doses of 10/30/100mg/kg IP	Gabapentin dose-dependently reversed mechanical hypersensitivity. The A_{50} (antinociceptive) dose was 26(16-42) mg/kg. The peak efficacy was observed 90 min after injection.	Estimated EC_{50} was higher than the administered dose range of 10-100mg/kg
4.	Whiteside *et al*(Whiteside *et al*., 2004)	Spinal nerve ligation rat model, clinical data	Comparison of human C_{max} at MED at daily maintenance dose (1800mg) to rat MED based on published literature	The concentrations at the rat MED (100mg/kg)was 191.54 nm compared to 69.72 nm at the human maintenance dose of 26 mg/kg	We estimated the EC_{50} at 43nm with a CV of 40%
5.	Yoon MA(Yoon and Yaksh, 1999)	FIP in rats	The antihyperalgesic effects of gabapentin (10, 30,100,300mg/kg) IP alone and in combination with ibuprofen (3, 10,30mg/kg) IP were tested. An isobolographic analysis was used to study the nature of the interaction.	The ED_{50} for gabapentin was 88mg/kg(51-141mg/kg, 95% CI) while that for ibuprofen was 19mg/kg (7–50, 95% CI)	Our EC_{50} was >100mg/kg

Table 6.4: Comparison of Experimental findings for gabapentin in various published preclinical and clinical studies. *(Continued)*

No.	Author	Experimental Model	Study Protocol	Main findings	Comparison with our work/ other remarks
			Comparison with Preclinical experiments		
6.	Hurley RW(Hurley *et al*., 2002)	Rat model of carrageenan induced inflammation	Gabapentin ,pregabalin or naproxen administered alone or in combination as oral gavage was administered to rats. An isobolographic analysis was used to study the nature of the interaction. Gabapentin was administered at does ranging from 3-300mg/kg	The ED_{50} for gabapentin was 19.2mg/kg(5.5-43.1mg/kg, 95% CI) while that for pregabalin was 6mg/kg (2.3–10, 95% CI) and for naproxen this was 0.48(0.05-1.38mg/kg)	In this animal model the ED_{50} for gabapentin was lower indicating higher potency as compared to that in our work
7.	Whiteside *et al*(Whiteside *et al*., 2004)	Rat model of incision pain	A number of analgesic drugs such as gabapentin, indomethacin and morphine were compared. Gabapentin doses were 10,30,100mg/kg	The MED for mechanical hyperalgesia was 30mg/kg, ED_{50} 11.3mg/kg For tactile allodynia the MED was 11mg/kg and ED_{50} 3.4mg/kg	In this animal model the ED_{50} for gabapentin was lower indicating higher potency as compared to that in our work
8.	Todorovic *et al*(Todorovic *et al*., 2003)	The radiant heat rat model of NP.	Anticonvulsants were injected intradermally into peripheral receptive fields of sensory neurons in the hind paws of adult rats, and paw withdrawal latency measured. Gabapentin (5-170µg), phenytoin (0.1-3 µg) , carbamazepine (0.1-2 µg), ethosuximide (140-1400 µg) were evaluated. Dose–response data were fit to the function $PI([-DRUG])=PI_{max}/(1+(ED_{50}/[DRUG])^n)$, where PI_{max} is the maximal percentage increase in PWLs caused by a drug in the injected vs. non-injected paw 10 min following injection, and n is the apparent Hill coefficient indicating the slope of the curve.	The ED_{50} was 80 µg/100ml or 4.67 nm	The 10 fold difference from our findings may be, in part, explained, in part, by different routes of administration in this study apart from a different experimental model and study setup.

Table 6.4: Comparison of Experimental findings for gabapentin in various published preclinical and clinical studies. *(Continued)*

No.	Author	Experimental Model	Study Protocol	Main findings	Comparison with our work/ other remarks
			Comparison with clinical/Translational experiments		
9.	Whiteside *et al*(Whiteside *et al*., 2004)	Spinal nerve ligation rat model, clinical data	Comparison of human C_{max} at MED at daily maintenance dose (1800mg) to rat MED based on published literature	The rat MED was 191.54nm compared to 69.72nm for humans	We estimated the EC_{50} at 43nm with a CV of 40%
10.	Lockwood *et al*(Lockwood *et al*., 2003)	A phase 3 study on patients with NP	The study was placebo controlled, double blinded. Patients were randomized to placebo or gabapentin treatments. A PKPD model (E_{max}) was fitted to data from patients with NP. The PKPD model was further used to simulate the MED for an investigational compound (pregabalin) based on in vitro potency information.	The EC_{50} estimated as 31.28nm (16% CV).	Correlates with EC_{50} estimated by us, however needs further confirmation.

APPENDIX

Pharmacokinetic concentrations

Gabapentin concentrations were obtained by simulation time points corresponding to those when pharmacodynamic measurements were recorded. A 2 compartment PK model with first order absorption was used for the simulations[16]. This model was based on data from two different experiments in Sprague-Dawley rats. In the first experiment, gabapentin was administered orally to conscious rats at doses of 0, 10, 100, 300 mg/kg in a formalin-induced hypersensitivity experiment similar to the current one, both of which ,in turn, are based on standard published experimental protocols.[2, 6]. Experimental groups consisted of three rats per dose level, with each animal contributing with four samples over a period of up to 6 h post-dose. The second experiment consisted of animals used in a microdialysis protocol receiving intravenous doses of 50mg/kg gabapentin (n=63). Each animal contributed with eight samples over a period of up to 24 h post-dose [42].

Published bioanalysis of gabapentin

Blood samples (100µl) were taken at the pre-defined time points up to 5 hours post-dose, namely 0, 2, 5, 15, 30, 60, 120, 180, 240 and 300 min. Plasma samples (50µl) were obtained by centrifugation at 4°C for 10 min and stored at -80°C until analysis. Gabapentin concentration in plasma was subsequently analysed by HPLC using pre-column derivatisation. Gabapentin and the internal standard 1-(aminomethyl) cycloheptaneacetic acid were allowed to react with 2,4,6-trinitrobenzenesulfone acid to form trinitrophenyl derivatives, which were then extracted with toluene, evaporated to dryness and reconstituted before injection. Analytes were resolved on a C_{18} reverse phase column using isocratic conditions. Mobile phase consisted of 58% acetonitrile in water containing 0.5% acetic acid. Ultraviolet absorbance was monitored at 35min. Quantification of the drug levels was based on the peak-height ratio. The lower limit of detection for gabapentin was typically 0.02µg ml^{-1} [43, 44]

Analytic Solution for the 2 compartment PK model-used for the PK simulations:

$$k_{el} = \frac{Cl}{V_1}, k_{12} = \frac{Q}{V_1}, k_{21} = \frac{Q}{V_2}$$

Where $k_{el=}$elimination rate constant (from the plasma compartment), $k_{12=}$micro-rate constant for transfer of gabapentin from the central to peripheral compartment, $k_{21=}$micro-rate constant for transfer of gabapentin from the peripheral to central compartment, V_1=central volume of distribution, V_2=peripheral volume of distribution, *Cl*=clearance from plasma, *Q*=intercompartmental clearance.

From the above the coefficient *A* may be calculated

$$A=k_{12}+k_{21}+k_{el}$$

From *A*, the two macro-constants or λ_1 and λ_2 *(*corresponding to the initial and terminal slopes representing bi-exponential decline respectively*)* may be further derived as follows

$$\lambda_1=\frac{A+\sqrt{A^2-4k_{21}k_{el}}}{2}$$

$$\lambda_2=\frac{A-\sqrt{A^2-4k_{21}k_{el}}}{2}$$

From these above the expression in equation 1 of the main text– for plasma concentrations is then derived.

$$C=\frac{k_aFD}{V_1}\left\{\frac{(k_{21}-\lambda_1)e^{-\lambda_1 t}}{(k_a-\lambda_1)(\lambda_2-\lambda_1)}+\frac{(k_{21}-\lambda_2)e^{-\lambda_2 t}}{(k_a-\lambda_2)(\lambda_1-\lambda_2)}+\left(\frac{(k_{21}-k_a)e^{-K_a t}}{(\lambda_1-k_a)(\lambda_2-k_a)}\right\}\right.$$

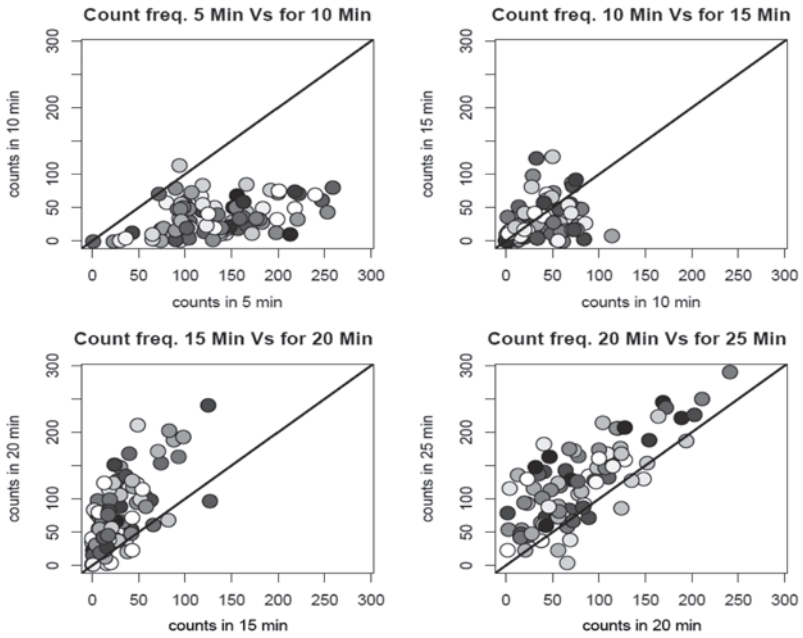

Figure 6.7: Correlations between observations at successive observation intervals. In the 4 panels, flinching counts at a particular interval are plotted against the corresponding counts in the next interval. Due to correlations between successive observations, the flinching patters show trends, towards decreasing frequency in the upper two panels and increasing in the lower panels.

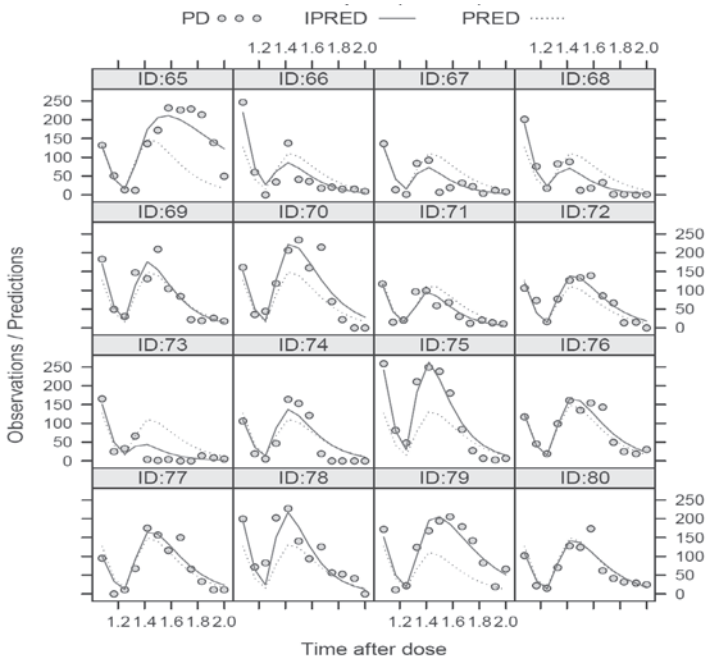

Figure 6.8: Example of randomly selected observed individual profiles (shaded circles) with the corresponding individual (IPRED, solid line) and population predicted (PRED, dotted line) response.

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