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

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SECTION III

LOST IN TRANSLATION-FROM BIOMARKER TO CLINICAL ENDPOINT

CHAPTER 7

Biomarker exposure-response relationships as the basis for rational dose selection: lessons from an ex-vivo model of inflammatory pain

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ABSTRACT

Lack of efficacy is one of the major causes of attrition in early clinical development. This is of particular concern in areas of high unmet medical need such as chronic inflammatory pain, where measures of efficacy cannot be quantified directly in healthy subjects. The evaluation and selection of an effective dose range for COX-inhibitors has been a matter of debate over the last decade. Yet, a systematic approach has not been fully implemented that enables the use of pharmacodynamics as a biomarker in a mechanistic manner to support the development of anti-inflammatory compounds. Here we apply PKPD modelling and simulation to assess the pharmacodynamic effects of a selective COX inhibitor across various clinically relevant scenarios and use biomarker response rather than drug exposure as the basis for dose selection in subsequent efficacy trials. Thereby, we are able to personalise and optimise the effective dose range in different patient sub-populations.

INTRODUCTION

A landmark study on attrition rates in drug development revealed that the major dropouts occur in Phase 2 and 3 [1]. In some therapeutic indications, such as chronic pain, such challenges are also observed during proof-of-concept (POC) studies. Potential explanations for these findings are species differences in target pharmacology or tissue distribution, poor tolerability due to inaccuracy in predicting the therapeutic index, insufficient target engagement, timing of the intervention relative to the onset of disease and trial design factors[2]. Whilst all the aforementioned factors can play a role in the results of a trial, target pharmacology and target (tissue) distribution have been considered a matter for basic rather than clinical pharmacology. Yet, they are essential for the characterisation of efficacy and safety and as such underpin the rationale for dose selection. In fact, as illustrated by Morgan *et al.*, development programs that have a positive readout at clinical POC also clearly express the pharmacology of the compound in humans [3]. Nevertheless, despite the increasing appreciation of the role of pharmacodynamic markers in clinical development, Phase 1 trials, including first-time-in-humans studies are typically designed to evaluate, systemic pharmacokinetics and tolerability[4]. These studies may be complemented by data from experimental models of pain, but measures of pain such as global pain scores are not integrated to pharmacokinetics or pharmacodynamics, and consequently, doses are selected without quantitative evidence about the extent and rate of target engagement [2]. These findings then become the reference for designing dose ranging studies in Phase 2 and defining the therapeutic dose(s) in Phase 3.

Undoubtedly, there is a pressing need to obtain early signals of efficacy and safety to prevent high attrition at late stages of development. This prerequisite is key for areas of high unmet medical need such as chronic pain and other immunoinflammatory conditions [5]. In these conditions inadequate decisions regarding dose selection during Phase 2a can propagate undetected into late development and have a disastrous impact on the life cycle of a novel molecule. Despite our evolving understanding of pharmacodynamics (PD), the evaluation of what constitutes a clinically relevant dose still relies primarily on empirical evidence, without any quantitative consideration of the underlying pharmacology or target engagement (e.g., receptor occupancy levels) in the patient population[3]. Currently, opportunities exist for truly characterizing the clinical pharmacological profile of novel molecules in humans, enabling mechanistic insight into the exposure-response relationships and consequently better rationale for the therapeutic dose range. Integration of biomarkers of pharmacology into drug development therefore becomes an opportunity to allow the implementation of the aforementioned concepts, eliminating part if not all the unobserved bias that arises from empirical evidence. Moreover, the assessment of pharmacokinetic-pharmacodynamic relationships based on biomarkers of pharmacology can provide a stronger basis for personalised medicine, which is often restricted to tailoring of treatment based on the

use of genetic information only[6]. By applying the mechanistic classification proposed by Danhof *et al*. [7], it is also possible to identify whether such relationships are drug or disease-specific and consequently to establish whether they can be used as predictive and prognostic tools during the development and therapeutic use of the drug.

In the current investigation we use data from a cyclo-oxygenase (COX) inhibitor to illustrate the concept of biomarker driven dose selection and emphasise the importance of gaining insight into the clinical pharmacology of the compound as the basis for the dose rationale and other relevant labelling information. The choice of the COX-2 system as a paradigm was dictated by the various reports arising from the withdrawal of different drugs from the market, for which the clinical pharmacology profile was known to determine efficacy and safety across different therapeutic areas, such as rofecoxib (2004), rimonabant (2008) and efalizumab (2009) [8-10]. Although complex interactions in mechanisms underlie the pathophysiology of chronic inflammatory conditions, the role of the COX-2 enzyme in the production of inflammatory mediators such as thromboxane B_2 (TXB_2) and prostaglandins (PG) has been clearly elucidated [11]. Selective COX-2 inhibitors are known to primarily inhibit PG synthesis [12].

Based on the aforementioned classification [7], PGE_2 and TXB_2 rank as biomarkers that reflect target engagement. Notably, the therapeutic dose range for chronic inflammatory pain for most non-selective and many of the selective COX-inhibitors has been defined according to empirical evidence of pain relief and analgesia after administration of discrete dose levels in clinical trials, regardless of the underlying pharmacology[13, 14]. It has been demonstrated, however, that pain relief appears to occur at PGE_2 inhibition levels of around 80%, i.e., complete suppression of COX-2 activity is not required to translate pharmacology into clinical improvement [15]. From these findings it can also be inferred that analgesia will also be observed at still higher levels of COX-2 inhibition, but such levels will lead to long term disruption of the normal physiological and homeostatic functions of the prostacyclin system, including tissue repair [16].

We use data from GW406381, an investigational and potent COX-2 inhibitor with demonstrated pre-clinical anti-inflammatory and analgesic activity [17, 18], to show that the study of such a mechanistic biomarker should be at the cornerstone of analgesic and anti-inflammatory drug development. The compound's pharmacokinetics as well as its effects on PGE_2 and TXB_2 were evaluated in an ascending dose study in healthy subjects, allowing the use of a biomarker-driven approach to select the doses for a Phase 2 study [19]. With the help of simulation scenarios we illustrate how biomarkers can be harnessed to explore the need for treatment personalisation (e.g., hepatic impairment) and quantitatively evaluate the rationale for the dosing regimen (e.g., optimised benefit-risk ratio).

METHODS

Clinical studies

Data from a human pharmacology study in healthy male subjects from the GSK (GlaxoSmithKline) clinical trial repository was used for the purposes of our analysis. This was a randomized, placebo controlled, double blind dose escalation parallel group study aimed at the evaluation of safety, tolerability, pharmacokinetics and pharmacodynamics of GW406381. Treatment consisted of a single dose followed by a 10-day repeated dosing phase (n=9 for the active and n=3 for the placebo arm). Data from placebo, 35 and 70 mg dose arms after the single dose phase and 35 mg dose arm after repeated dosing were used in our analysis. The study was conducted according to the principles of good clinical practice (GCP) and the declaration of Helsinki pertaining to research on human subjects [20, 21]. All subjects provided their written informed consent for participation and the study was approved by the Institutional ethics committee. Further information on subject demographics and the study protocol is provided in Table 7.3 (see appendix).

Pharmacokinetic-pharmacodynamic modelling

To guide the model building, exploratory analysis was carried out by plotting the time course of the biomarker levels as well as the drug concentration *vs.* biomarker levels profile. The PKPD analysis was subsequently carried out sequentially in two steps, with modelling of PKPD data after completion of the pharmacokinetic analysis. Details on the pharmacokinetic modelling can be found in the appendix to this manuscript. All modelling was performed in NONMEM®, version 7.2 (Icon, Dublin Ireland), using the FOCE (first order conditional estimation) method. PsN 3.5.3 was used to run NONMEM, whilst data manipulation and plots were performed in R 2.13[22].

Both for the PK and PKPD analysis, a parameter Θ for an individual *i* was described by the following expression:

$$\Theta_i = \Theta_{TV} * exp^{\eta i} \quad (1)$$

Where Θ_{TV} is the typical (population) value of the parameter, η is a random variable with zero mean and a variance ω^2.

Inter-individual variability (IIV) was parameterised using an exponential distribution model. The square root of the variance is reported for IIV, as this is an approximation to the apparent coefficient of variation of a normal distribution on log-scale. The residual variability comprising measurement and model misspecification errors was described with an exponential model, thus for

$$Y_{ij} = F_{ij} * exp^{\varepsilon ij} \quad (2)$$

Where ***Y*** is the j^{th} observed concentration in the i^{th} individual.
F is the predicted concentration and ε is a random variable with zero mean and variance σ^2. The concentration-biomarker response relationships were described by the following equation/expression for the sigmoid I_{max} model

$$Eff = I_0 - (I_0 - I_{max}) * \frac{C^{\gamma}}{C^{\gamma} + IC^{\gamma}_{50}} \quad (3)$$

Where I_{max} represents the maximum inhibitory response to GW406381 plasma concentrations (C), I_0 is the baseline production of PGE_2 and γ is the Hill factor. The covariate effects of baseline PGE_2 on the parameter I_0 was tested according to the following expression

$$I_{0i} = I_{0TV} * \frac{BAS_i - MED}{MED} \quad (4)$$

where I_{0i} represents the parameter value for the i^{th} individual, I_{0TV} is the population value of the parameter, BAS_i and MED represent the individual and median values of the baseline PGE_2, respectively.

Model evaluation and validation procedures

Parameter inclusion and thus final model selection was based on the likelihood ratio test, parameter point estimates and their respective 95% confidence intervals (CI) as well as goodness of fit plots. For the likelihood ratio tests, the significance level was set at 0.01 which corresponds with a decrease of 6.63 points after the inclusion of one parameter in the minimum value of the objective function (MVOF) under the assumption that the difference in MVOF between two nested models is χ^2 distributed. Visual goodness of fit plots comprised individual *vs.* population or individual predictions, and weighted residuals *vs.* time or population predicted values. Minimisation was considered successful in case the minimisation occurred with a positive covariance step and no associated error messages.

Validation

The precision of estimated model parameters was assessed using a non-parametric bootstrap. Two thousand bootstrap samples were generated in PsN 3.5.3 [23]. Results were used to assess model stability and obtain estimates for the coefficient of variation for relevant model parameters. The mean and standard errors of the parameters obtained from bootstrapping were subsequently compared with those obtained by fitting the model to the original dataset. Finally visual predictive checks were used to visually inspect the concordance between simulated data and real observations. Using the final model parameters, 2000 datasets were simulated and the simulated data overlaid with the real observations.

Simulations

The last part of this work was to simulate analgesic doses in patients based on biomarker inhibition data from human subjects, under the assumption that pharmacodynamics in the target population are comparable, other than differences in baseline levels of inflammatory mediators due to differences in disease conditions. Most importantly, it was assumed that the analysis was based on the premise that PGE_2 inhibition represents a causal step in the pain cascade [24]. The drug effect was parameterised in terms of IC_n as per the following expression [15].

$$IC_n = \left(\frac{n}{100-n}\right)^{\frac{1}{\gamma}} * IC_{50} \quad (5)$$

where n = the degree or extent of COX-2 inhibition in percentage.

Using the final PKPD model, concentration-biomarker response profiles were simulated for a number of clinical scenarios are presented in Table 7.1 below.

Table 7.1: Factors altering drug exposure

Scenario	Altered parameters	Remarks	Reference
Liver dysfunction	Cl: -25% Cl: -50% Cl: -75%	Mild, moderate and severe liver dysfunction-as per Child Pugh scores	Schmitt-Hoffmann *et al*, 2009[25]
Systemic vasculitis (General inflammation)	I_0: +30% I_0: +50%	Significant interferon inhibitory activity attributed to increased levels of soluble interferon receptors, PGE_2 levels and interferon inhibitory protein	Ambrus JL, *et al.* 1997[26]
CYP3A4 induction	Cl: +25% Cl: +50%	Induction of CYP3a4	Maronpot *et al.* 2009[27] Hewitt *et al.* 2007[28]
Combination of liver dysfunction and general inflammation	Cl: -25% - I_0: +30% Cl: -50% - I_0: +50%	Lower clearance with general inflammation	Vet, N *et al.* 2011[29]
Once vs. twice daily dosing	None	The same regimen was compared as once vs. twice daily doses	NA

For each scenario, concentrations at steady state were generated for 50 subjects per dose group, assuming treatment for two weeks using a q.d. regimen. The dose range used for these scenarios was 0, 20, 35, 70, 100, 150, 250 and 400 mg. Samples were collected on the first and last treatment day before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24 h after administration.

The simulated scenarios were based on the anticipated clinical relevance (i.e., safety and efficacy), of different levels of COX inhibition. A summary of the simulation algorithm is depicted in Figure 7.1.

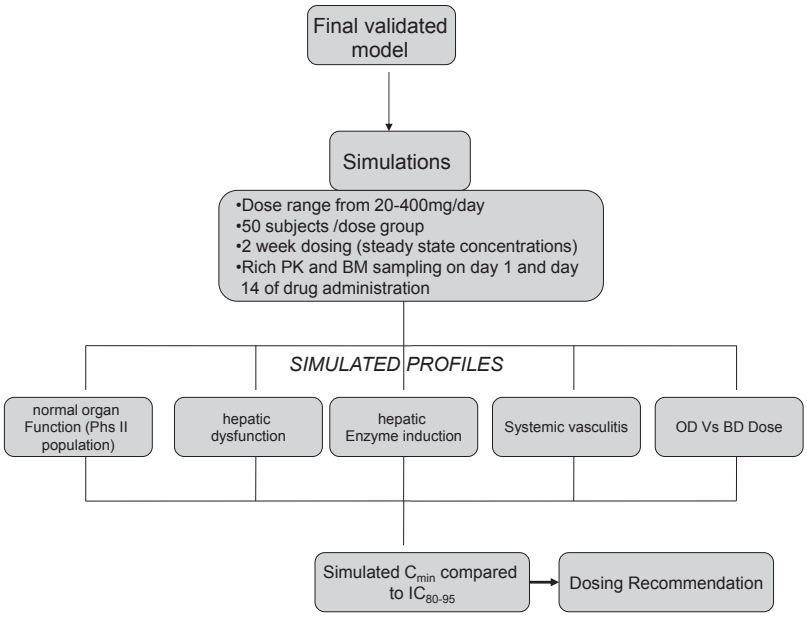

Figure 7.1: Diagram depicting the simulation protocol. Five different clinical scenarios were simulated; each having the characteristics shown in the box above the simulated profiles. The simulated trough (C_{min}) concentrations were compared to the estimated IC_{80-95} to assess deviations from the putative therapeutic window.

The simulations were also used to calculate the putative therapeutic windows at dose level. For each dose, trough concentrations at the steady state (C_{min}) were simulated and compared to the benchmark values, namely IC_{80} (efficacious levels), IC_{90} (maximum desirable response) and IC_{95} (potential safety risk). A ratio of 1 represented optimum desired concentrations while IC_{90}/C_{min} of 2.5 was assumed to be the upper margin of the therapeutic window. At concentrations of around or greater than the IC_{95}, safety events were assumed to be expected. Therefore, effective but non-toxic doses were defined as those at which the C_{min} values at steady state were around the IC_{80}, while the C_{max} was below the IC_{95}. In addition, given that for meaningful analgesic response, i.e., not only the attainment but also the maintenance of pain relief is important, the time span during which drug concentrations remained within the therapeutic window (i.e., between IC_{80} and IC_{95}) was evaluated for twice daily dosing and compared with the standard q.d. regimen.

RESULTS

Pharmacokinetic Analysis

A two-compartment model with first order absorption and elimination best described the PK of GW406381 in adults. Due to high variability in the data, higher concentrations were

found to be slightly under predicted. This discrepancy may be due to the absence of data on influential covariates, which means that not all of the observed variability could be fully characterised. Nevertheless, interindividual variability (IIV) was identified on the peripheral volume (V3), clearance (CL), absorption rate constant (ka) and bioavailability (F_1). Residual variability was best described using an exponential error model. The PK parameters from the final model as well as the results of a non-parametric bootstrap are presented in Table 7.2.

Table 7.2: Final pharmacokinetic and pharmacodynamic model parameter estimates and the results of a non-parametric bootstrap (n=2000).

Model estimates			Bootstrap Results		
Parameter	**Final Model estimates**	**CV%**	**Median**	**%5 CI**	**95% CI**
V_2 (L)	252.38	35.06	242.93	160.39	416.84
V_3(L)	959.78	60.54	954.25	476.81	2115.03
CL (L/h)	30.21	43.97	29.89	17.86	58.61
K_a (h^{-1})	15.24	78.7	10.83	3.92	35.81
Q (h^{-1})	37.28	35.82	35.6	23.83	62.44
Lag time (h)	0.47	6.05	0.47	0.41	0.49
F_1 35	1.00	*FIXED*	*FIXED*	*FIXED*	*FIXED*
F_1 70	0.49	44.2	0.48	0.25	0.93
IIV V_3	93%	73.05	98%	30%	145%
IIV CL	56%	76.4	53%	32%	84%
IIV K_a	198%	48.84	166%	98%	258%
IIV F1	95%	50.58	91%	53%	133%
Residual error	0.11	58.99	0.1	0.05	0.23
PD parameters			**PD Bootstrap Results**		
I_0 (pg/ml)	63196.80	9.74	63077.60	53861.30	73871.50
I_{max} (pg/ml)	479.00	*FIXED*	*FIXED*	*FIXED*	*FIXED*
IC_{50} (ng/ml)	43.25	12.22	43.69	36.04	53.21
Hill factor	1.59	10.37	1.61	1.42	1.94
IIV I_0	44%	28.63	42%	33%	54%
IIV I_{max}	272%	107.38	241%	0%	343%
Residual error	0.002	10.91	0.002	0.001	0.002

Where V_2 and V_3 =central and peripheral volumes of distribution respectively, Cl=clearance from the central compartment, Q=intercompartmental clearance,F_1=relative bioavailability

Although the coefficient of variation for some of the parameter estimates was high, all findings were comparable to the bootstrap median results except for Ka, which showed 50% higher variation than the bootstrap median. The observed profiles and model fits are presented in the supplemental material (Figure 7.6), along with diagnostics of individual

and population predictions as well as an individual predicted profile. The high IIV was reflected in the visual predictive check. The medians of the predicted and observed data are very similar, however, the uncertainty around the predictions, is maximum above the 95% quartile, especially in the multiple-dose-phase for the 35 mg dose group (see Figure 7.7).

PKPD analysis

GW406381 did not have any effect on TXB_2 levels. Therefore, only PGE_2 data were analysed. The I_{max} model was fitted using the PK parameters estimated during the PK analysis. The PKPD model was able to describe the data adequately, as assessed by the basic goodness of fit plots (see right panel Figure 7.6, in the appendix). High variability was seen in the baseline (I_0) PGE_2, which is most conspicuous in the placebo group. Interindividual variability was modelled exponentially and IIV identified only on two parameters. Not all subjects showed high concentrations due to highly variable exposure, whereas maximum PGE_2 inhibition was observed in only 3 subjects. A summary of the PD parameters from the final model along with the estimates from the nonparametric bootstrap estimates are presented in Table 7.2. From the visual predictive checks, it can be seen that variability is inflated at the upper boundary of the confidence interval (see Figure 7.7 in the appendix).

Simulation scenarios

We have assumed that effective analgesic and anti-inflammatory effects could be achieved and maintained when PGE_2 inhibition are kept above 80%, but below 95%. Based on the predicted potency estimates, a range of doses from 20-400 mg/day was investigated. From a physiological perspective, two parameters were considered to fluctuate in the target population, depending on intrinsic or extrinsic factors, such as differences in metabolism and disease conditions, namely *CL* and I_0.

Patients with Normal Organ Function

Patients with normal organ function were used as a reference for the other scenarios. The objective of this scenario was to provide the range of doses which provide clinically relevant target engagement. As can be observed from the concentration *vs.* time profiles, the median peak concentrations lie below the IC_{95} after a dose of 100 mg daily. Biomarker response increases in a nonlinear manner, with trough concentrations reaching IC_{80} values after 250 mg given as a once daily dose regimen. However, at this dose level peak concentrations are above IC_{95}. Consequently, to remain within the proposed therapeutic range (i.e., $\geq$80% and < 95% inhibition), the median effective therapeutic dose appears to lie between 70 mg and <250mg/day. (See Figure 7.2).

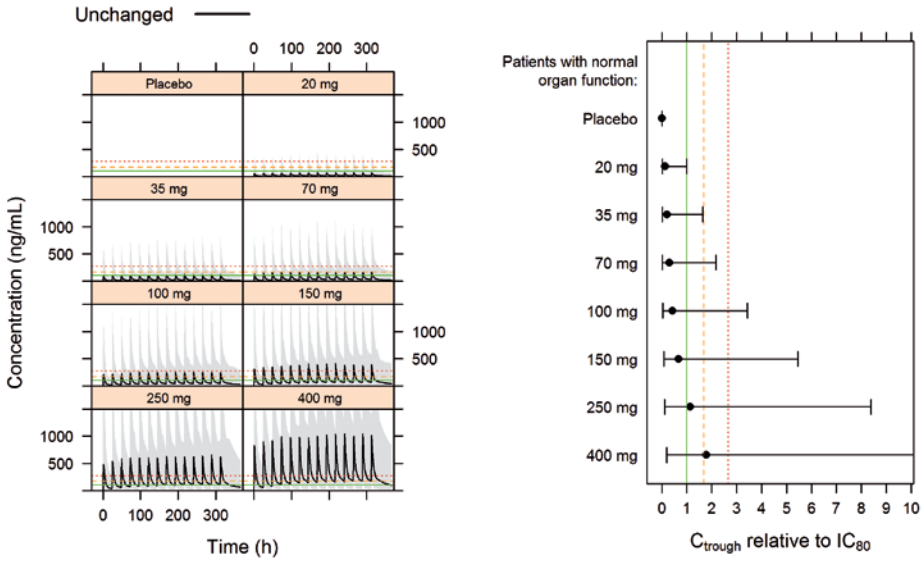

Figure 7.2: Patients with normal organ function. (Left panel*)* GW406381 concentration vs. time stratified by dose group. Black solid line depicts median concentrations in patients with reference parameter values whilst the shaded area represents the 90% confidence interval (right panel). Forest plots showing drug response associated with different dose levels. X-axis shows the concentration at trough, relative to the IC_{80} of GW406381 stratified by dose group. The solid black circles depict median concentration whilst solid lines represent the 90% confidence interval. Green solid line: IC_{80}. Orange dashed line: IC_{90}. Red dotted line: IC_{95}.

Patients with hepatic impairment

Given the metabolic elimination route (CYP3A4) of GW406381, patients with hepatic impairment are likely to show decreased clearance of the drug. In this scenario, we explored how changes in clearance alter drug exposure and consequently biomarker response. Patients with severe liver impairment needed a longer time to reach steady state (>5 days), especially in the higher dose groups. Furthermore, the trough concentrations were found to be higher than IC_{95} values for the dose groups receiving >250mg. The forest plot (Figure 7.3) reveals that the median trough concentration reached the IC_{80} for the mild, moderate and severe forms of liver impairment at doses of 150, 100 and 35 mg, respectively. Based on these findings, the doses of GW406381 to be used in mild hepatic impairment should be between 100-150mg, whilst for moderate and severe impairment further reductions should be considered (i.e., from 70-100 mg and 20-35 mg respectively).See Figure 7.3.

Metabolic (CYP3A4) induction

The dose required to reach IC_{80} trough concentrations was higher in this scenario, as compared to patients with normal organ function. GW406381 concentrations were overall lower, and steady state concentrations were reached soon after start of the treatment.

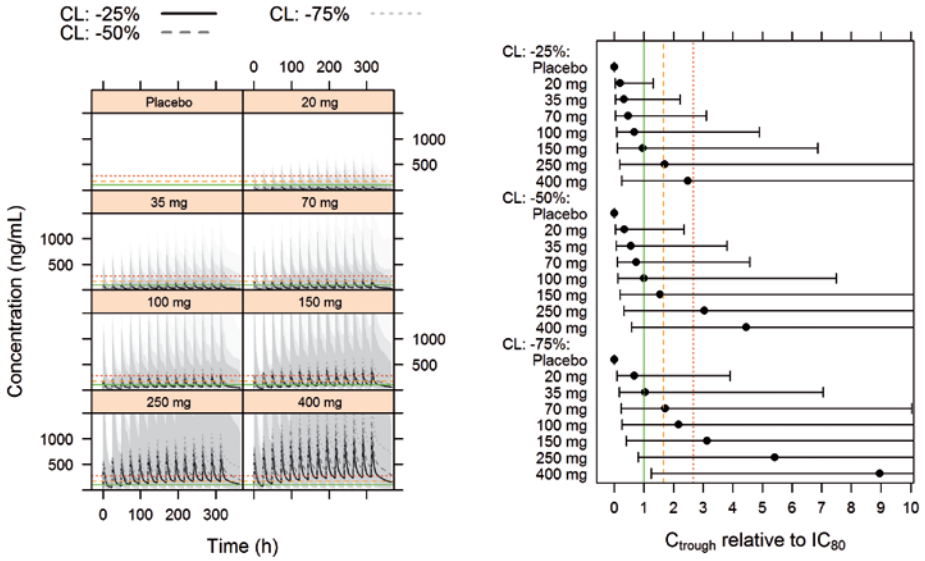

Figure 7.3: Patients with decreased liver function. Panel (a-left) GW406381 concentration vs. time stratified by dose group. Black solid line depict median concentrations in patients with hepatic impairment, whist the shaded area represents the 90% confidence interval. Panel (b-right) Forest plots showing the different dose levels. X-axis indicates drug concentrations at trough, relative to the IC_{80} of GW406381 stratified by dose group. Percentages reflect the predicted change (%) in hepatic function. Solid black circles: median concentration. Solid lines: 90% confidence interval. Green solid line: IC_{80}. Orange dashed line: IC_{90}. Red dotted line: IC_{95}.

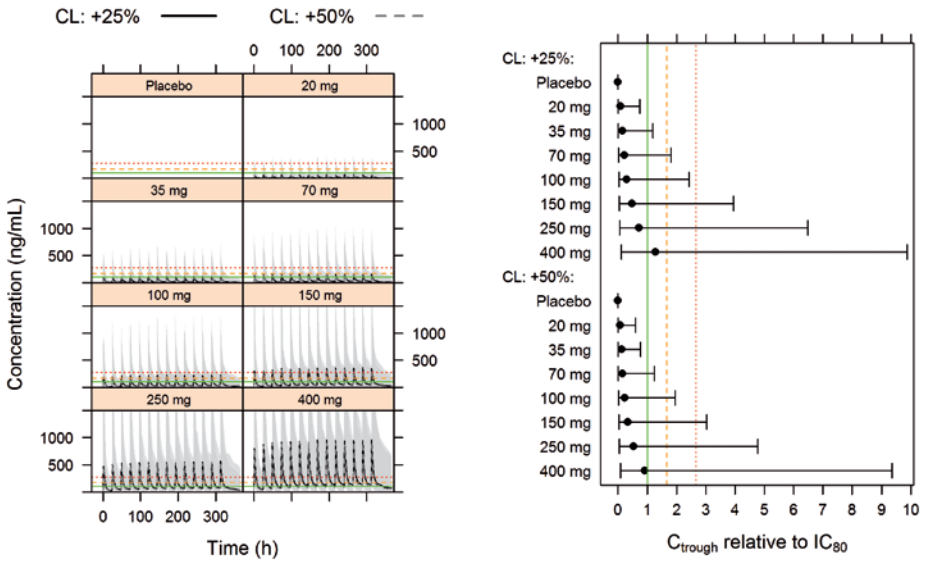

Figure 7.4: Metabolic enzyme (CYP3A4) induction, with 25% CL and 50% increase in CL. Panel (a-Right) Concentration vs. time stratified by dose group. Panel (b-Left) Forest plots showing the different dose levels. Percentages reflect the predicted change (%) in hepatic function.

The difference between 25% and 50% increase in clearance are negligible, as evident from the forest plots. The overall trough concentrations relative to the IC_{80} are lower than what was observed in the reference groups with normal organ function. IC_{80} values are reached at trough levels for doses between 250 and 400 mg when total clearances increased by 25%. Median plasma concentrations of GW406381 were below IC_{80} values throughout the simulated dose range when 50% increase in clearance was considered. See Figure 7.4.

Inflammatory conditions

A scenario was considered in which symptoms worsen as compared to standard inflammatory diseases such as in systemic vasculitis or generalised septicaemia. Interestingly, the dose range required to yield effective exposure in these patients remained the same as in patients with normal organ function. Elevations in baseline PGE_2 did not appear to alter the concentration-effect relationships. Consequently, these findings indicate that no dose adjustment is required in patients showing variable degrees of inflammatory response.

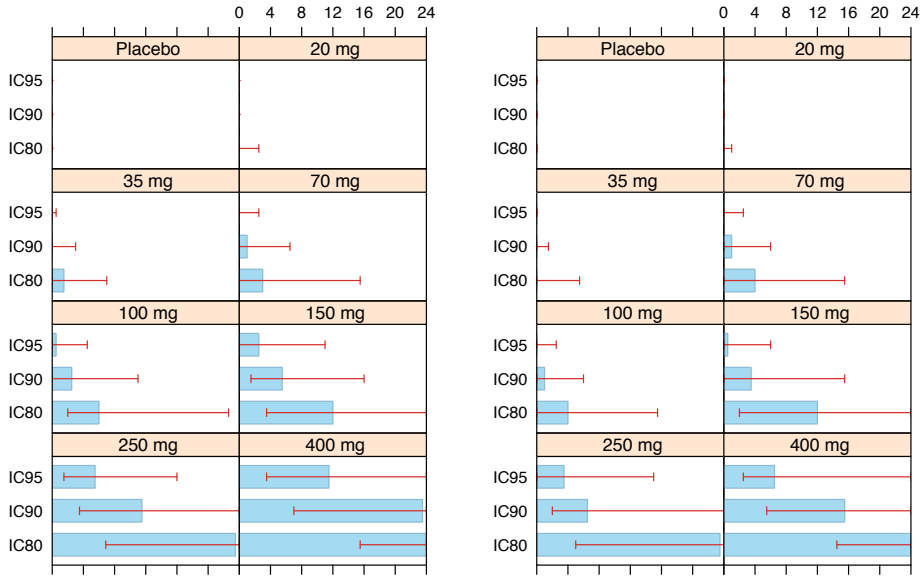

Figure 7.5: Time above the $IC_{80, 90, 95}$ respectively for once daily (OD, left panel) and twice daily (BID, right panel) stratified by dose group, in patients with normal organ function. Red bars depict the 5 and 95% Confidence intervals respectively.

Once daily (q.d.) vs. twice daily (b.i.d.) dosing regimens

Given the proposed therapeutic range (i.e., ≥80% and < 95% inhibition), it was found that a b.i.d. regimen allowed peak concentrations to remain above the IC_{95} for a shorter time and at much higher dosages, without significant effect on trough concentrations, which were

comparable to those achieved with an q.d. regimen. Furthermore, important differences can be noticed in the total time plasma concentrations remain above IC_{80}, IC_{90} and $IC_{95.}$ Whilst no differences are observed for the ratio between trough concentrations to IC_{80}, the total time above IC_{95} was significantly lower (see Figure 7.5).

DISCUSSION

The rationale for the therapeutic dose range has always been a difficult aspect of drug development. Recommended doses and dosing regimens are often defined early on in development when information on the drug's pharmacology is scarce. As can be deduced from the number of drugs for which the recommended dose has undergone revisions, dose selection remains a point of concern in even in the post marketing phase[30]. The possibility of generating data on a compound's pharmacology represents an opportunity to optimise and personalise treatment during the development programme.

Phase I studies have traditionally been designed with the aim of evaluating the maximum tolerated dose (MTD) in humans [4, 31] . Even though the scope of these studies has expanded in recent years to allow the early evaluation of pharmacodynamics, challenge models in healthy subjects, similar to animal models of pain, reproduce symptoms rather than expressing the pharmacology of the compound[32]. In fact, their translational relevance is questionable. Data from these models have been documented to be non-specific and can at times yield contradictory results [33].

Conceptually, dose selection and optimisation of pain control have been primarily determined by techniques such as titration to effect [13]. In addition, subjective scales have been endorsed as clinical endpoints of choice for the evaluation of analgesia in regulatory guidance documents for neuropathic as well as nociceptive pain[34, 35].Not surprisingly, there is little evidence in the published literature of clinical trials in which the dose selection for appropriate analgesia has been based on pharmacological activity. Here we have illustrated the concept of biomarker driven dose selection and emphasise the importance of gaining insight into the clinical pharmacological properties of a compound to ensure accurate assessment of safety and efficacy early in the clinical development programme. Specifically, we show how such biomarkers can be used in a quantitative manner to guide the dose selection and identify the conditions requiring dose adjustment. With the help of simulation scenarios, we show how the scope of Phase I studies may be expanded to understand the pharmacology of candidate compounds, taking into account different characteristics of target sub-populations, who would be likely recipients of the drug later in the clinical development programme.

From a methodological perspective, even in circumstances where high variability exists in the data, the use of a model-based approach in conjunction with biologically relevant

model parameterisation allows one to explore the impact of individual differences in pharmacokinetics and pharmacodynamics and quantify the overall consequences (i.e., uncertainty or true interindividual variation) of variability on dose selection for different groups in the target patient population. In our example, the variability in the actual data was attributable firstly to the hepatic metabolism of GW406381, as it is a CYP3A4 substrate [17, 36]. It is well known that CYP3A4 substrates show high IIV in metabolism [37]. Secondly, considerable IIV has been reported in the degree of COX-2 inhibition and selectivity in similar assays of enzymatic activity in healthy subjects [38]. The high variability observed in healthy subjects also exposes a limitation of using *in vitro* potency as a benchmark to compare compounds in early clinical development, which does not reflect differences in selectivity or metabolic activity *in vivo*. In fact, Fries *et al.* showed that despite the higher potency of rofecoxib relative to celecoxib *in vitro*, their *in vivo* selectivity is likely to be the same [38]. Likewise, the *in vitro* potency of GW406381 was estimated to be approximately 30 times as high as rofecoxib[39]. However, the optimal recommended dose range proposed from our simulations lies between 150-250 mg, while that for rofecoxib is 25-50mg[40]. This is mostly explained by the inter-individual differences in pharmacokinetics and enzyme activity described above.

We acknowledge that such an exercise presumes the availability of biomarkers of pharmacology, which may not always be readily measurable in a different disease or therapeutic indication. Yet, there are some general principles of basic pharmacology that can be extended to clinical pharmacology studies, i.e., that target engagement determines therapeutic response and as such needs to be taken into account for the purposes of dose selection [2, 3]. Evidence of clinical efficacy and safety without further characterisation of the underlying pharmacological activity is misleading. Of particular relevance in the case of COX-inhibitors for the treatment of chronic inflammatory pain is the fact that clinical response is reached below maximum target engagement [24, 41]. Similarly, the use of target engagement or target receptor occupancy may be applied to the evaluation of various other drugs (e.g., antibodies, cannabinoids and centrally acting analgesics), subject to the availability of a suitable mechanistic biomarker of response.

In chronic pain, hypersensitivity is the result of downstream effects of COX-2 production which, in turn, is mediated by PGE_2 and TXA_2 [11, 42]. However, pain scales are considered pre-requisites for demonstrating evidence of analgesic and anti-inflammatory response ,[43, 44]. Various issues arise from such an empirical, fragmented approach to drug development; the most important one being the inability to define the true therapeutic window. By contrast, the use of a biomarker-driven approach provided us insight into the therapeutic window. Under the assumption that drug exposure levels leading to > 95% inhibition in biomarkers (i.e.,IC_{95}) is above the therapeutic margin, we could show how the risk of adverse events (AEs) can be mitigated by ensuring drug levels at doses yielding exposure

within IC_{80}-IC_{95} values. This therapeutic window is based on the investigation of Huntjens *et al.* who have shown that analgesic therapeutic plasma concentration is directly correlated with IC_{80}[45]. At COX-2 inhibition >90%, treatment effects suppress the physiological levels of COX-2, which are also present under healthy conditions. In addition, for some drugs, COX-2 selectivity may also be lost, which would then result in adverse events associated with COX-1 inhibition. This subtle balance has been highlighted by Capone *et al.* who have shown that a correlation exists between COX-2 inhibition greater than 90% and elevated risk of cardiovascular events [46]). Clearly, our work illustrates how the pharmacodynamics of this class of compounds can be used as a proxy or predictor of clinical response.

In contrast to traditional non-steroidal anti-inflammatory drugs, selective COX-2 inhibitors do not alter TXB_2 levels, which act as a pro-coagulant [12, 47]. In fact, concerns about the safety of selective COX-2 inhibitors arise from the pharmacological activity on its primary target [48-51]. As can be seen from our simulations, the reported cardiovascular events with this class of compounds is likely to be the result of an inappropriately high dose, the selection of which was not based on pharmacological activity, but rather on the statistical significance of the differences between active and placebo treatment arms [52-55]. McGettigan *et al.* have proposed that there exists gradient of cardiovascular risk for COX-2 inhibitors which runs from protective to risk-inducing, i.e., lower doses are cardio protective, becoming risk-inducing at higher doses[56]. Furthermore, additional evidence points to a time-dependent effect, suggesting that it's the prolonged suppression of COX-2 activity that may ultimately determine adverse cardiovascular outcome [57].

Lastly, we have attempted to show how different dosing regimens affect the therapeutic window in clinical practice. Our analysis reveal that optimal exposure to GW406381 can be achieved by constraining drug concentrations to fluctuate within the range comprised between IC_{80} and the IC_{95} values. Such a requirement can be met by the administration of GW406381 according to a twice daily dosing regimen. This finding can be partly substantiated by the safety profile of celecoxib, which is also prescribed as b.i.d. regimen [58].

Methodological Limitations

Our exercise had some limitations, which for the sake of clarity are worth mentioning. We assumed that PGE_2 inhibition is required not only for the onset but also the maintenance of pain response. The role of secondary, downstream mediators known to contribute to the inflammatory process has been excluded from our analysis [59]. In addition, the data available for this exercise did not include any other intrinsic factor or covariate that might contribute to further changes in response to COX inhibition, such as differences in receptor density or other mediators that might antagonise the effects of COX-2 inhibition. We have also assumed that the disease status and processes do not alter during the time span considered for the simulation scenarios. However, it has been shown that in certain conditions, such as

systemic inflammation (vasculitis or rheumatoid arthritis), other circulating mediators such as cytokines vary over time and may therefore influence pain response over time [60].
Another obvious criticism is the lack of prospective validation of the simulation scenarios and availability of data confirming the suggested dose recommendations. As this is the crux of matter in terms of the concepts implemented here, we refer the reader to a few examples from published literature in which population-based approaches have been used for dose selection and extrapolation purposes [61-63]. The most compelling example is provided by the work of Huntjens *et al.* With the help of PKPD modelling, they have analysed human *in vitro* and *ex vivo* PGE_2 inhibition data and were able to demonstrate that IC_{80} estimates for fenoprofen were similar between healthy subjects and patients with systemic lupus erythematosus (SLE)[64]. Subsequently, based on simulations the authors conclude that doses above 600-800 mg/day yield concentrations above IC_{80} for at least 80% of the dosing interval (24hrs). This compares favourably with the recommended total daily analgesic fenoprofen dose of 800-1200 mg for the relief of mild to moderate pain in adults [65]. The common denominator in all these examples is that the biological substrate across the populations or experimental groups is the same.
In conclusion, the role of biomarkers expands beyond the potential diagnostic and prognostic value currently perceived by most investigators in industry and academia. In contrast to many of the translational efforts using pre-clinical species [66], biomarkers offer a mechanistic basis for the characterisation of PKPD relationships and as such provide valuable guidance for the dose selection as well as for the design of subsequent studies during drug development. Moreover, this approach contributes to further dismantling of an entrenched belief that still pervades the field of clinical pharmacology, i.e., that the maximum tolerated dose should be evaluated in subsequent efficacy trials, irrespective of any evidence of underlying target engagement.

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Conflicts Of Interest

Oscar Della Pasqua is an employee of GlaxoSmithKline UK. The authors have no other conflicts of interest.

Author Contributions

AT and SPO conducted the research, which was planned and conceptualised by ODP. MD and ODP were involved in the manuscript preparation along with AT and SPO.

Table 7.3: Demographic and Study Protocol Information on Study Subjects.

Study Phase	No of Subjects	Subject Demographics	Dose/s Administered	PK sampling Times	PD sampling times
Single Ascending Dose (day 1)	2 cohorts of 12 subjects each.	A healthy adult male in the age ranges 18-45. Weight range 55-95kg. BMI 19-29kg/m^2	In Cohort 1, subjects were randomized to receive 35mg(n=9) or matching placebo (n=3), while in cohort 2 the subjects received 70mg or placebo.	Pre-dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48 and 54 hrs post-dose.	PGE_2 Pre-dose and 2, 4, 6, 8, 12, 24,48 hrs post-dose TXB2 Pre-dose, 2, 4, 6, 8, 12, 24,48 hrs post-dose
DRUG FREE PERIOD UPTO DAY 9					
Multiple dose (day 9-18)	1 cohort of 12 subjects	as above	35mg(n=9) or matching placebo, once daily from day 9-18	At steady state on day 18 Pre-dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48 and 54 hrs post-dose.	At steady state on day 18 PGE_2 Pre-dose and 2, 4, 6, 8, 12, 24,48 hrs post-dose TXB2 Pre-dose, 2, 4, 6, 8, 12, 24,48 hrs post-dose

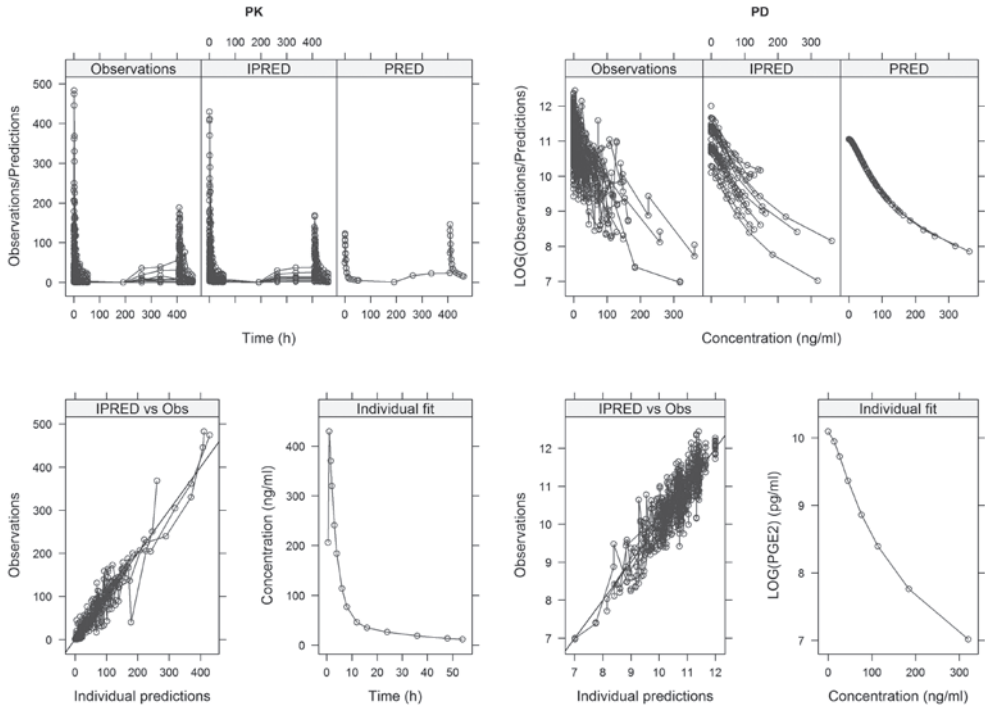

Figure 7.6: Diagnostics (goodness-of-fit plots) for the pharmacokinetic (left panels) and pharmacokinetic-pharmacodynamic (right panels) models. The upper panels show the observed data, the individual and population predictions vs. time (PK panels) or concentration (PD panels). The lower left graph shows the observed data vs. individual predictions, whereas the lower right graph depicts an individual predicted profile.

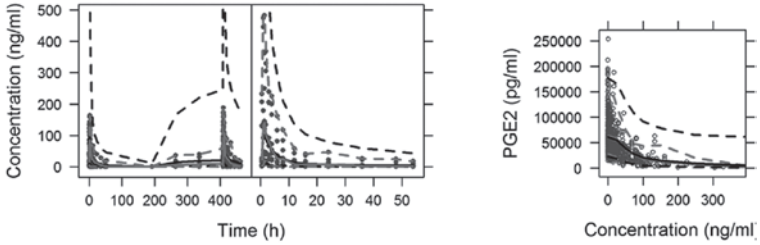

Figure 7.7: Visual Predictive checks of final pharmacokinetic model (left panels) and PKPD model (right panel). The dots represent the actual observations, whereas the lines represent the median (solid line) and the 5th and 95th percentile (dashed line) of the real data. For the VPC on the right, the black lines represent the 5th, 50th and 95th percentile of the simulated data, respectively.

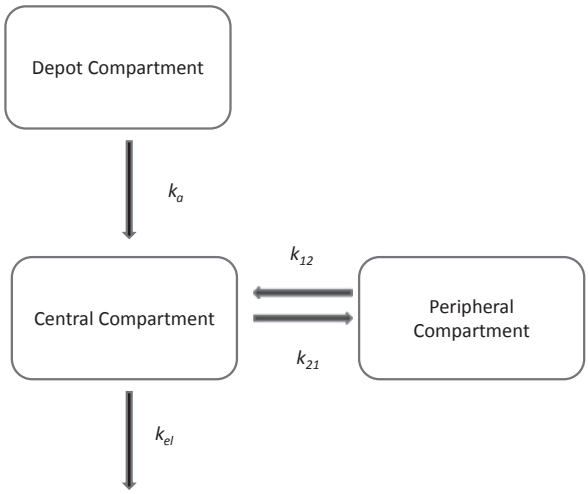

Figure 7.8: Two-compartment pharmacokinetic model with first order absorption used to describe the pharmacokinetics of GW406381 in plasma.

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