Beyond relief : biomarkers of the anti-inflammatory effect and dose selection of COX inhibitors in early drug development

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Chapter 7

Impact of chronic inflammation on the pharmacokinetic-pharmacodynamic relationship of naproxen

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ABSTRACT

Objectives. The use of biomarkers for predicting the clinical doses relies on the understanding of the relationship between drug exposure and response under disease conditions. In this study, we demonstrate the relevance of such a relationship for COX inhibitors by modelling the effect of naproxen on prostaglandin E2 (PGE_2) and thromboxane B2 (TXB_2) in a chronic inflammation model in rats.

Methods. Rats were treated with Freund's complete adjuvant (FCA) by intraplantar injection. On post-inoculation days (PID) 7 to 21, animals received single or chronic (QD until day 21) doses of naproxen (10 mg/kg). Blood samples were collected at various intervals after dosing to characterise naproxen pharmacokinetics and its effects on PGE_2 and TXB_2 production. PKPD modelling was performed using nonlinear mixed effects in NONMEM.

Results. The inhibition of PGE_2 and TXB_2 could be described by a sigmoid Emax model. A decrease in the potency estimates of both biomarkers was observed under chronic inflammation, as compared to healthy animals. IC_{50} values for PGE_2 inhibition showed a shift from 2840 ± 510 to 4000 ± 677 ng/ml (mean ± SD), whilst IC_{50} values for TXB_2 inhibition increased from 1180 ± 323 to 3360 ± 453 ng/ml in healthy and FCA-inoculated animals, respectively.

Conclusions. Our results show that chronic inflammation causes a significant change in the potency estimates for COX-inhibition. These findings illustrate the implications of pathophysiological processes on pharmacodynamics and consequently on the required exposure levels for achieving response during chronic treatment.

INTRODUCTION

Blockade of cyclo-oxygenase (COX) activity by cyclo-oxygenase inhibitors results in direct suppression of the formation of pro-inflammatory mediators such as prostaglandins (PG) and thromboxanes (TXB) (1). In fact, the selectivity of action with regard to COX isoforms has substantiated the extensive use of these drugs to achieve analgesia in acute and chronic inflammation. Yet, the screening and dose rationale for such compounds remain empirical, in that dose estimates are usually derived from behavioural measures in animal models for which understanding of the underlying inflammatory processes is rather limited. This approach presupposes that behavioural measures are sufficient evidence to support the selection of doses in clinical studies, irrespective of the extent and duration of effect on biomarkers of inflammatory response. Hence, no effective evaluation has been performed to establish the correlation between biomarkers in these models of inflammation and human inflammatory conditions.

From a clinical pharmacology perspective, systematic ways to identify dosing regimens that produce clinically relevant analgesia should be in place during pre-clinical drug development (2). An important question that needs to be answered is how much and how long COX-2 and COX-1 should be inhibited to ensure an optimal risk-benefit ratio, allowing for a sustained analgesic response and an appropriate safety margin in the treatment of chronic inflammatory conditions. Unfortunately, at present the dose selection of COX inhibitors is primarily based on evidence from clinical analgesia, disregarding whether maximum, long-lasting blockade of either enzyme systems is strictly required for response (3). In fact, continuous COX-1 inhibition is known to induce gastrointestinal adverse effects, rather than analgesia following prolonged administration of non-selective COX inhibitors. On the other hand, selective sub-maximal COX-2 inhibition accounts for the anti-inflammatory and analgesic efficacy (4), it is likely that prolonged suppression of COX-2 activity may underlie the cardiovascular events observed after chronic administration of selective COX-2 inhibitors. In that sense, the use of a biomarker in pain measurements may represent an important step in the development of new compounds, enabling bridging of pharmacokinetics to the analgesic effect as well as providing a proxy for safety. Moreover, it would facilitate the extrapolation of pre-clinical findings *in vivo*, yielding earlier and more accurate predictions of the required doses in phase I and II studies (3).

Given the nature of the inflammatory response and the mechanism of action of COX inhibitors, primary candidates for biomarkers of analgesia are PG and TXB. Using a model-based approach, it is possible to establish the relationship between these biological markers, pain measurement and safety. In this respect, we have recently shown the value of PKPD modelling to quantitatively characterise the inhibition of TXB_2 and PGE_2 by naproxen *in vitro* and *in vivo* in rats and healthy human volunteers (5). In the current investigation, we evaluate the impact of chronic inflammation on the effect of COX inhibitors on PGE_2 and TXB_2 and its implications for dosing regimen in chronic treatment conditions. We attempt to address the aforementioned question using naproxen as a paradigm compound in the Freund's complete adjuvant (FCA) model of chronic inflammation in rats.

MATERIALS AND METHODS

Animals. The experimental protocol was approved by the Ethical Committee on Animal Experimentation of the University of Leiden. Experiments were performed on male Sprague-Dawley (SD) rats (Charles River B.V., Maastricht, The Netherlands) weighing 322±5 g (Mean ± SEM, n=34). The animals, six per cage, were housed in standard plastic cages with a normal 12-hour day/night schedule (lights on 07.00 a.m.) and a temperature of 21^{0}C. The animals had access to standard laboratory chow (RMH-TM; Hope Farms, Woerden, The Netherlands) and acidified water *ad libitum*.

Drug administration, Naproxen (Sigma Alldrich BV, Zwijndrecht, The Netherlands) was dissolved in sterile 0.9% NaCl solution and administered intraperitoneally as a bolus at a dose of 10 mg/kg.

Experimental model of inflammation. Freund's complete adjuvant (FCA) (100 µl of a 1 mg/kg M. tuberculosis (1 mg/kg), Alldrich BV, Zwijndrecht, The Netherlands) was injected intraplantarly with a 27-gauge needle into the left hind paw. The FCA injection produced an area of localised erythema and oedema that did not disturb grooming, sleep-wake rhythm or social interactions. Animals were divided into two groups for evaluation of acute and chronic treatment effect.
The first group of animals (acute treatment) were treated with a single i.p. dose of naproxen at post -inoculation day (PID) 7 and PID 21 (10 mg/kg), whilst the second group of animals (chronic treatment) received daily i.p. doses of naproxen (10 mg/kg) from PID 7 until PID 21. Naproxen was given in a dose volume of 1 ml/kg. Blood samples of 250 µl were taken from the tail vein at pre-defined time points for the determination of drug, TXB_2 and PGE_2 concentrations. Blood was drawn prior to FCA administration and PID 1, 7 or 8 and 14 or 15 for the determination of PGE_2 and TXB_2 concentrations under acute and chronic inflammatory conditions. Sampling from the tail vein was limited to 7 blood samples per animal on the experiment day. Experiments were started in the morning (08.00 a.m.) or in the evening (06.00 p.m.) to account for eventual circadian variation in pharmacodynamics. Prior to drug administration, a baseline measurement was obtained to assess biomarker levels and drug concentrations at day 7 and day 21. Six post-dose samples were taken for the experiments starting in the morning, namely between 3-20 min (sample 1), 3-5 h (sample 2), 6-8 h (sample 3), 8-11 h (sample 4), 11-14 h (sample 5) and approximately 24 h (sample 6). Six post-dose samples were taken for the experiments starting in the evening, namely between 3-20 min (sample 1), 14-16 h (sample 2), 16-18 h (sample 3), 18-20 (sample 4), 21-23 h (sample 5) and approximately 24 h (sample 6). Blood samples were split into aliquots of 100 µl (for PK and PGE_2) and 50 µl (for TXB_2). Blood samples for PK were placed into heparinised tubes and centrifuged at 5000 rpm for 10 min. Plasma was stored at -20^{0}C until analysis. Blood samples for TXB_2 analysis were placed into tubes and allowed to clot for 1 hour at 37^{0}C in a stirring water bath. Serum was collected after centrifugation and stored at -20^{0}C until analysis. Tubes for the analysis of PGE_2 were prepared by evaporating aspirin (10 µg/ml in methanol and heparin (10 I.U.) Blood samples were placed in tubes and 10 µg/ml lipopolysaccharide (LPS) was added. Samples were incubated and stirred for 24 hours at 37^{0}C in a water bath. Plasma was separated by centrifugation and stored at -20^{0}C until analysis.

Drug analysis. Drug concentrations were analysed according to the method described by Satterwhite and Boudinot (6). Briefly, plasma samples were spiked with 50 µl of internal standard (1000 mg/ml ketoprofen in methanol). The pH was adjusted by addition of 0.2 ml 1M phosphate solution at pH 2. After extraction with 5 ml diethyl ether, the residue was dissolved in 100 µl mobile phase, of which a volume of 50 µl was injected into the HPLC system. The HPLC system consisted of a Waters 501 Solvent pump, a Waters 717plus autosampler (both Millipore-Waters, Milford, MA, USA), Superflow 757 Kratus UV absorbance detector (Shimadzu, Kyoto, Japan). Chromatography was performed on a C18 3 µm cartridge column (100 x 4.6 mm i.d., Chrompack, Bergen op Zoom, The Netherlands) equipped with a guard column. The mobile phase consisted of 0.02 M phosphate buffer (pH 7.0) and acetonitrile (82:18 v/v) with a flow rate of 1 ml/min. Detection was achieved by measuring the ultraviolet absorbance at a wavelength of 258 nm. Data acquisition and processing was performed using a Chromatopac C-R3A integrator (Shimadzu, Kyoto, Japan). The signal showed linearity over the range of 50-100000 ng/ml. The within- and between-day coefficients of variation of the assay were 1.82% and 8.21%, respectively.

Analysis of TXB_2 and PGE_2. PGE_2 and TXB_2 were measured by a validated enzyme immunoassay (EIA) (Amersham Biosciences Europe GmbH, Freiburg, Germany). Briefly, samples were diluted in assay buffer (2-50 times for PGE_2, 200-2000 times for TXB_2) and a 50 µl sample was transferred into a coated well plate. After addition of 50 µl antibody and 50 µl peroxidase conjugate, samples were incubated for 1 hour, washed four times and incubated for 15 min (TXB_2) or 30 min (PGE_2) after which 150 µl substrate was added. The enzyme reaction was halted by addition of 100 µl 1M sulphuric acid and optical density was measured in a plate reader at 450 nm.

Data analysis. The pharmacokinetics and pharmacodynamics of naproxen were assessed by nonlinear mixed effects modelling, as implemented in NONMEM version V, level 1.1 (Globomax, Ellicott City, USA). Modelling of TXB_2 and PGE_2 inhibition was based on sparse pharmacokinetic and pharmacodynamic sampling in non-cannulated animals under acute and chronic inflammatory conditions. To ensure accurate estimation and identifiability of random effects on relevant model parameters, data published previously by Huntjens *et al.* on the pharmacokinetics and pharmacodynamics of naproxen in healthy animals were included in the analysis (5). Final model parameters were estimated by the first order conditional estimation method with η-ε interaction (FOCE interaction). This approach allows the estimation of inter- and intraindividual variability in the model parameters. All fitting procedures were performed on a computer (AMD-Athlon XP-M 3000+) running under Windows XP with a Fortran compiler (Compaq Visual Fortran, version 6.1). An in-house interface for S-Plus 6.0 (Insightful Corp., Seattle, WA, USA) - NONMEM was used for data processing, management and graphical display.

Naproxen pharmacokinetic analysis. A standard two compartment pharmacokinetic model published by Huntjens *et al.* was used for the simultaneous analysis of data presented in this paper (5). Due to practical limitations, no plasma samples could be collected during the absorption phase

after intraperitoneal injection. Given the exceptionally rapid absorption rate, this problem was circumvented by treating the absorption phase as intravenous data.

The influence of chronic inflammation by FCA on naproxen disposition was tested by estimating different fixed and random parameters for healthy and disease conditions. Model selection and identification was based on the likelihood ratio test, parameter point estimates and their respective 95% confidence intervals, parameter correlations and goodness-of-fit plots. For the likelihood ratio test, the significance level was set at $p< 0.01$, which corresponds with a decrease of 6.6 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. The following goodness-of-fit plots were subjected to visual inspection to detect systemic deviations from the model fits: individual observed vs. population or individual predicted values and weighted residuals vs. time or population predicted values.

Based on model selection criteria, a two compartment model was identified to describe the pharmacokinetics of naproxen under healthy and disease conditions. The pharmacokinetic analysis was performed by use of the ADVAN6 routine in NONMEM. Model parameterisation included clearance (CL), inter-compartmental clearance (Q), and volumes of distribution in the central (V_1) and peripheral compartment (V_2). Variability in pharmacokinetic parameters was assumed to be log-normally distributed in the population. Therefore an exponential distribution model was used to account for inter-individual variability:

$$P_i = \theta_i \cdot \exp(\eta_i) \quad (1)$$

where θ is the population estimate for parameter P, P_i is the individual estimate and η_i is the normally distributed between-subject random variable with mean zero and variance ω^2. The coefficient of variation (CV%) of the structural model parameters is expressed as percentage of the root mean square of the interindividual variance term. Selection of an appropriate residual error model was based on inspection of the goodness-of-fit plots. A combination of a proportional and an additive error model was then proposed to describe residual error in the plasma drug concentration:

$$C_{obs,ij} = C_{pred,ij} \cdot (1 + \varepsilon_{ij,1}) + \varepsilon_{ij,2} \quad (2)$$

where $C_{obs,ij}$ is the j^{th} observed concentration in the i^{th} individual, $C_{pred,ij}$ is the predicted concentration, and ε_{ij} is the normally distributed residual random variable with mean zero and variance σ^2. The residual error term contains all the error terms that cannot be explained by other fixed effects including experimental error (e.g. error in recording sampling times) and structural model misspecification.

Pharmacodynamic analysis. As no specific covariate was found for pharmacokinetics, population parameter estimates were used as input for estimating plasma concentration at the sampling times for pharmacodynamics.

PGE_2 and TXB_2 concentrations are used in this study as a measure of drug response. The sigmoid

I_{max} model was used to relate naproxen plasma concentration (C) to the drug response by the equation:

$$Effect = I_0 - (I_0 - I_{max}) * (C^n / (C^n + IC_{50}^n)) \quad (3)$$

where I_{max} represents the maximal inhibitory response to naproxen, I_0 the baseline production of PGE_2 or TXB_2 and n the Hill coefficient. This equation is an adaptation from the E_{max} model in order to obtain the absolute values for I_0 and I_{max} for the direct calculation of maximal inhibition in percentages.

Exploratory graphical analysis showed a correlation between clock time and I_{max} for PGE_2 inhibition, which was described by the following equation:

$$I_{max} = \theta_i + \theta_j \cdot clock\ time \quad (4)$$

where I_{max} is the maximal inhibitory response, θ_i and θ_j are intercept and slope of model parameter I_{max} versus clock time relationship, respectively.

Covariates. The role of potential covariate factors on model parameters was screened by univariate analysis. Covariates were then incorporated into the model by stepwise forward inclusion. A significance level of $p<0.01$ was used for inclusion, which represented a drop of least 6.63 units in the objective function for each additional parameter A final evaluation of the statistical significance of all factors identified during the previous step was performed by subtracting each covariate individually (backward elimination). The final structural model (i.e., fixed effects model) included only those covariates whose subtraction resulted in a decrease of at least 3.84 units in the objective function ($p<0.05$)

Visual predictive check. The performance of the population PK and PKPD models were assessed by simulating 1000 data sets with the final model parameter estimates. The mean and the 95 % confidence intervals were calculated for naproxen, PGE_2 and TXB_2 concentrations at the pre-defined sampling time points for the PK and PKPD models, respectively.

Statistical analysis. Comparisons of the observed responses between pre- and post-FCA injection of PGE_2 and TXB_2 productions were made by a paired t-test. Statistical significance was set at $p<0.05$.

RESULTS

Baseline biomarkers. A summary of the systemic levels of biomarkers before and at various days after FCA inoculation is shown in Table 1. No significant differences were observed in PGE_2 ($p=0.66$) and TXB_2 ($p=0.32$) levels before and after FCA injection.

Pharmacokinetics. A two-compartment model with combined proportional and additive errors best described the pharmacokinetics of naproxen under healthy and disease conditions. The time course

of observed and predicted naproxen concentrations is depicted in Figure 1. Additional modelling diagnostic plots reveal no bias or trends that might propagate into the PKPD analysis, for which predicted naproxen concentrations were used (Figure 2).

Table 1. Baseline biomarker levels before and after FCA inoculation in rats.

Biomarker concentration (ng/ml)*	Baseline**	PID 1	PID 7	PID 14	PID 21
PGE_2	79±33	89±18	84±33	75±40	52±27
TXB_2	365±209	390±240	514±307	406±271	341±296

The only difference found between healthy and FCA-innoculated rats was an increase in the volume of distribution (V_1). The influence of potential disease processes on pharmacokinetics was investigated and an increase in the central volume of distribution was found in disease status after single dose administration (46.1 ml in healthy rats and 75.1 ml in diseased rats). After chronic administration, the central volume of distribution was further increased to 166 ml. Pharmacokinetic model parameter estimates are summarised in Table 2. Clearance was found to be independent of the

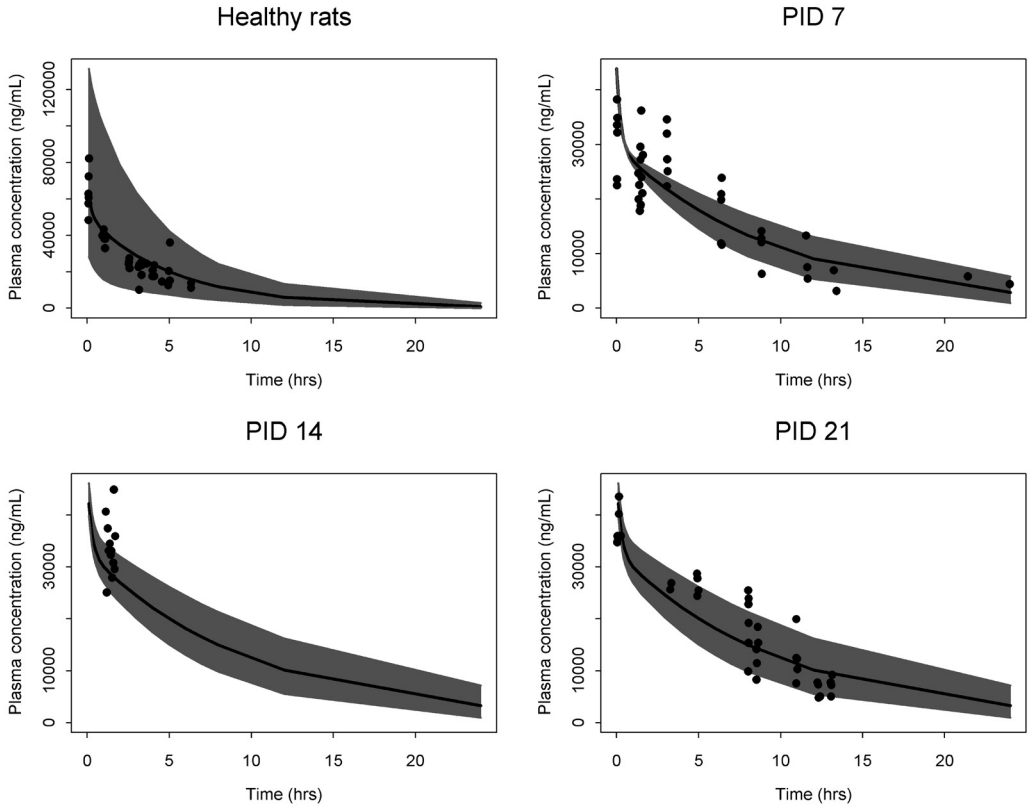

Figure 1. Population pharmacokinetic profiles of naproxen (10 mg/kg i.p.) in healthy and chronic disease conditions. Solid symbols represent individual data points. Grey area indicates 95% confidence intervals. Black line represents the population prediction. Upper left graph: naproxen exposure in healthy rats. Other panels: naproxen exposure in chronic inflammation separated by PID.

disease status. Inter-individual variability for clearance could be estimated in healthy and disease conditions, but only in healthy rats for volume of distribution. A correlation was observed between ω^2Cl, ω^2V_1 and ω^2V_2 and therefore the covariance of those parameters was added to the final model. The correlation coefficients were 0.81 for ω^2Cl and ω^2V_1 ($p < 0.001$), 0.57 for ω^2Cl and ω^2V_2 ($p<0.001$) and 0.40 for ω^2V_1 and ω^2V_2 ($p<0.001$).

PKPD relationships. Large variability was observed in the systemic levels of PGE_2 and TXB_2. Nevertheless, maximum inhibition of PGE_2 and TXB_2 was immediate, with trough values being achieved at the first sampling time point after administration of naproxen. The PKPD relationship was best described using a sigmoid I_{max} model. The population and individual predictions together with observed PGE_2 and TXB_2 inhibition are depicted in Figure 3. Diagnostic plots of model fitting are shown in Figure 4.

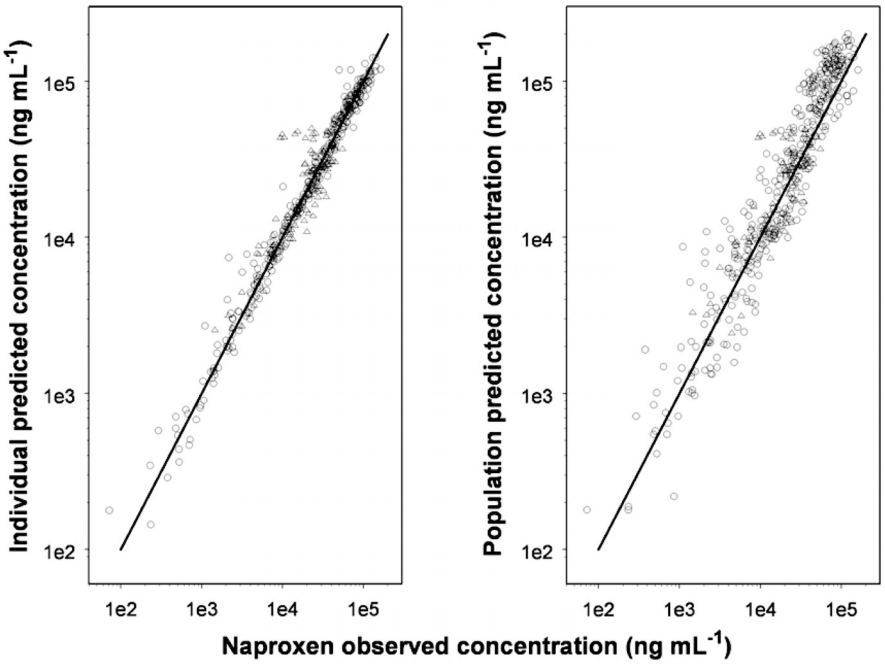

Figure 2. Diagnostic plots for the population pharmacokinetic model of naproxen (10 mg/kg i.p.) in healthy and chronic disease conditions. Symbols represent individual data points for healthy animals (open triangles) and FCA-inoculated rats (solid triangles). Solid line indicates the line of unity. A: observed versus predicted population concentrations.
B: observed versus predicted individual concentrations.

Table 2. Population pharmacokinetic estimates of naproxen in healthy and disease conditions. Values between parentheses are coefficient of variation.

	Final Model Estimates Naproxen	
	Fixed effects	*Random effects*
Cl (mL/h)	21.7 (18)	19 (66)
Q (mL/h)	103 (18)	
V_1 (mL) Healthy	48.5 (8)	42 (17)
V_2 (mL) Healthy	28.2 (17)	76 (22)
V_1 (mL) FCA	75.7 (8)	
V_2 (mL) FCA	37.9 (15)	
Residual variability		
Proportional error (%) Healthy	17 (17)	
Proportional error (%) FCA	36 (18)	
Additive error FCA (ng/mL)	178 (59)	

Whilst baseline levels of biomarkers were found to be unaltered under disease conditions, drug effect on PGE_2 and TXB_2 was significantly different between healthy and disease conditions. For PGE_2 inhibition different estimates were obtained for potency, Hill coefficient and baseline PGE_2 in healthy and FCA-inoculated rats (Table 3). For TXB_2 inhibition, potency, Hill coefficient and maximal inhibition differed between healthy and disease conditions (Table 3).

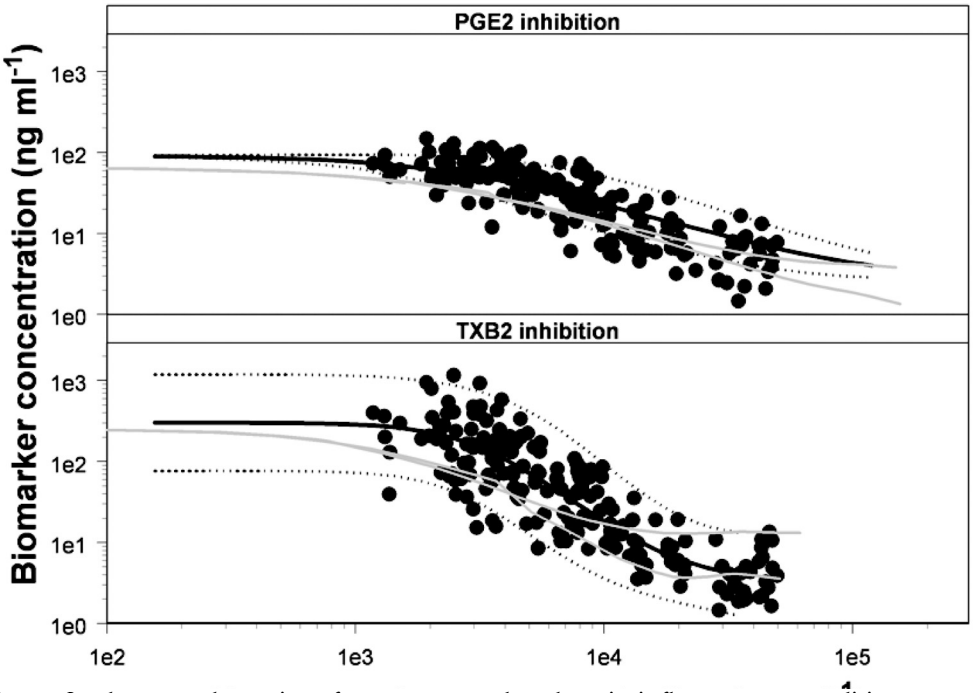

Figure 3. Pharmacodynamics of naproxen under chronic inflammatory conditions. Upper panel: naproxen exposure versus PGE_2 concentrations. Lower panel: naproxen exposure versus TXB_2 concentrations. Solid symbols represent individual data points. Solid black line indicates the population predicted response in chronic inflammation, dashed lines represent 95% confidence intervals (n=36 rats). Grey lines represent the population predicted naproxen exposure in healthy animals (two lines are shown, one for an experiment started in the morning and one for an experiment started in the afternoon, based on the effect of clock-time as covariate for healthy rats).

Inter-individual variability was found on IC_{50} for PGE_2 inhibition and on IC_{50}, I_{max} and I_0 for TXB_2 inhibition. All parameters were accurately estimated as indicated by their CV%. IC_{50} estimates for PGE_2 inhibition in healthy and FCA-inoculated rats were 2840 ± 510 and 4000 ± 677 ng/ml (mean ± SD, p=0.25), respectively. IC_{50} estimates for TXB_2 inhibition in healthy and diseased rats were 1180 ± 323 and 3360 ± 453 ng/ml ($p<0.05$), respectively. The maximum inhibitory effects of naproxen resulted in more than 95 % suppression of PGE_2 and TXB_2. In addition, exploratory graphical analysis showed a correlation between clock time and I_{max} for PGE_2 inhibition, indicating the influence of circadian variation on this parameter. The incorporation of clock time as a function of I_{max} significantly improved the fit ($p<0.001$). A clock time cycle of 24 hours was defined with zero being set at 08.00 a.m. In contrast, there was no correlation between clock time and baseline levels of PGE_2. Other procedural and time-related covariates such as time after FCA injection (PID 7 versus PID 21) were not statistically significant.

Given the degree of COX-inhibition required for analgesic and anti-inflammatory effects of COX inhibitors, IC_{80} values were also calculated. The IC_{80} values for PGE_2 inhibition were 8534 and

Table 3. Population pharmacodynamic estimates for the *in vivo* inhibitory effects of naproxen on PGE_2 and TXB_2 production in rats. Values in parentheses are relative standard errors (%) of the estimates.

Parameter	Parameter estimate	Parameter estimate
	Naproxen	
Fixed effects	*PGE_2 inhibition*	*TXB_2 inhibition*
I_0 (ng mL^{-1}) Healthy	64.5 (4)	290 (12)
I_0 (ng mL^{-1}) FCA	91.2 (7)	290 (12)
IC_{50} (ng mL^{-1}) Healthy	2840 (18)	1180 (27)
IC_{50} (ng mL^{-1}) FCA	4000 (17)	3360 (13)
Hill coefficient Healthy	1.26 (10)	1.39 (15)
Hill coefficient FCA	1.01 (11)	2.77 (10)
I_{max} (ng mL^{-1})		
$\theta_{Intercept}$	2.67 (12)	
θ_{Slope}	-0.265 (10)	
I_{max} (ng mL^{-1}) Healthy		12.0 (21)
I_{max} (ng mL^{-1}) FCA		3.1 (14)
Random effects		
ω IC_{50} (%) Healthy	103 (32)	60 (45)
ω IC_{50} (%) FCA	42 (28)	29 (37)
ω I_0 (%) Healthy		38 (67)
ω I_0 (%) FCA		65 (35)
ω I_{max} (%) FCA		63 (37)
Proportional error (%) Healthy	44 (8)	43 (11)
Proportional error (%) FCA	44 (8)	61 (15)

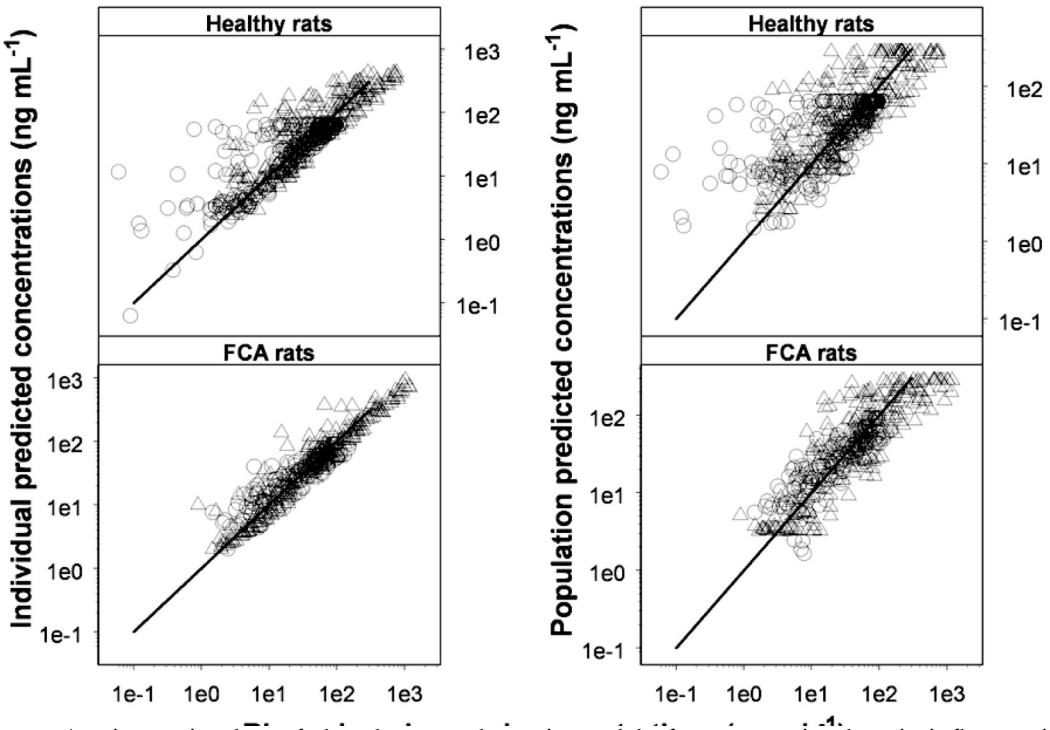

Figure 4. Diagnostic plots of the pharmacodynamic model of naproxen in chronic inflammation. Symbols represent individual PGE_2 and TXB_2 concentrations for healthy animals (open triangles, open squares) and FCA-inoculated rats (solid triangles, solid squares), respectively. Solid line indicates the line of unity A: observed versus predicted population concentrations. B: observed versus predicted individual concentrations.

15782 ng/ml for healthy and FCA-inoculated rats, respectively. IC_{80} values for TXB_2 inhibition were 3199 and 5542 ng/ml for healthy and FCA injected rats, respectively.

DISCUSSION AND CONCLUSION

In the current study we have investigated the impact of chronic inflammatory conditions on the PKPD relationships of naproxen as a paradigm compound. The inhibition of PGE_2 and TXB_2 was selected as pharmacodynamic endpoint and biomarker of drug effect *in vivo*. These endpoints are proposed as basis for the selection of clinical dosing regimen instead of the currently accepted behavioural measures of pain and inflammation, an approach which may enable accurate interspecies scaling of anti-inflammatory response and provide a proxy for the reputed toxicity associated with the chronic use of COX inhibitors.

Pharmacokinetics. Thus far, few publications have discussed the pharmacology of analgesic drugs in animals and tried to correlate to clinical findings (7;8). Yet, none of these investigations have considered the impact of pharmacokinetics or disease processes on such a correlation. The present study was designed to assess the impact of inflammatory conditions on the pharmacological response to naproxen in animals and correlate our results with clinical findings taking into account and excluding confounding by pharmacokinetic factors.
The differences in volume of distribution observed in FCA-inoculated rats may not be relevant for drug activity. In fact, the increase in volume of distribution can be explained by the increase in blood vessel wall permeability due to inflammation. We have also found larger residual variability in the FCA treated rats (17% in healthy rats versus 36% in FCA rats), which could be due to the disease condition itself. Interestingly, thorough suppression of PGE_2 and TXB_2 was observed at concentrations higher than 50 μg/ml. This exposure is equivalent to analgesic plasma levels in humans achieved after a 500 mg dose of naproxen (9).

Our results also clearly show that whilst systemic exposure is not altered upon chronic dosing with naproxen, pharmacodynamic changes do occur, which cannot be attributed to pharmacokinetic factors. Similarly, differences exist in the dosing regimen for analgesia in chronic pain conditions (e.g., osteoarthritis and rheumatoid arthritis) in humans, which are unrelated to the pharmacokinetics of naproxen (10).

Inflammatory markers and COX-activity under disease conditions. The relevance of systemic markers of inflammation depends upon whether COX-activity in plasma can be linked to the target tissue and whether drug disposition does not differ significantly between tissues. In our experiments, local levels of inflammatory mediators have not been assessed. However, the absence of significant differences in the blood levels of PGE_2 and TXB_2 before and after FCA inoculation does not suggest that inflammation is not systemic. In this sense, we acknowledge that the use LPS to stimulate monocytes in blood samples *ex vivo* to produce PGE_2 may hamper the detection of changes in PGE_2 concentrations due to inflammation itself.

On the other hand, the rationale for dosing regimen with an anti-inflammatory drug must take into account that the inflammation is a dynamic process and various mediators are up and down-regulated at different time-points, yielding variation in drug response due to changes in the underlying inflammatory conditions (11). Such changes are also known to influence pain processing and subsequently cause behavioural measures of hyperalgesia and allodynia to vary over time. In this context, one may need to reconsider the emphasis on COX-2 as the inducible enzyme playing a major role in hyperalgesia. The existence of COX-1 in areas and tissues associated with nociceptive response and the fact that it can also be induced means that a role for COX-1 is far from ruled out. The data available would tend to suggest a role for COX-1 in acute nociception, with inducible COX-2 mechanisms prevailing in chronic conditions. Such activity explains the efficacy of selective COX-1 inhbitors (12).

Another important consequence of the use of biomarkers for dose selection is the ability to account for the relative contribution of different targets to the overall analgesic effect, which could enhance the development of drug combinations for chronic pain conditions. An example of this approach has recently been highlighted by Kortotkova *et al.* who have investigated how local treatments with glucocorticoids led to a significant down-regulation of mPGE synthase as well as COX-1 and COX-2. Most importantly, the use of biomarkers circumvents the lack of sensitivity of behavioural measures of pain, which rely on experimental readouts that are insensitive to differences in pharmacokinetics and pharmacodynamics or may not be relevant to the target mechanism (18).

PKPD modelling and disease processes. In pain research, considerable weight is given to extrapolation of the results obtained in pre-clinical models of acute pain without much attention to the impact of chronic diseases on drug effect (13). In addition, results are often expressed in terms of effective doses (e.g., ED_{50}) per unit body weight, which makes scaling between species very empirical.

In contrast, the use of biomarkers as basis for predicting the analgesic dose in humans may facilitate further understanding of adverse events associated with chronic use of NSAIDs, which often cannot be fully characterised in pre-clinical safety and toxicology experiments. Moreover, it prevents misinterpretation of experimental results due to the lack of sensitivity of endpoints and inaccuracy due to poorly designed *in vivo* experiments.

Recently, Whiteside *et al.* have evaluated the predictive validity of pain models by comparing the pharmacokinetic-pharmacodynamic relationship for pain drugs in rats and humans (14). A discrepancy of up to 4 fold is observed between species for drugs used in inflammatory pain and up to 37 fold for neuropathic pain. However, their findings do not provide further understanding of how differences in pharmacological properties determine subsequent target activation and downstream response in either species. In addition, they fail to explain the variation in dose requirements for efficacy in chronic pain conditions (14).

We anticipate that the characterisation pharmacokinetic-pharmacodynamic relationships for markers of inflammatory response may contribute to overcoming the empiricism in dosing rationale for treatment of pain. In particular, PKPD relationships can be extremely useful if parameterisation of experimental results takes into account the pharmacological nature of the drug-receptor interactions, for instance by assessing IC_{80} instead of IC_{50} for enzyme inhibitors. In fact, the relevance of IC_{80} estimates from the human whole blood assay *in vitro* to predict the therapeutic analgesic dose in patients has been highlighted previously (3). The advantages of such an approach can be exemplified by our current findings with naproxen. On a dose basis, empirical comparison with other COX inhibitors show large difference between estimates from animal data and clinically prescribed doses (see Table 4).

Such discrepancies are difficult to interpret without further evidence of how each drug actually interacts with the target and to what extent the downstream cascade is being inhibited. The importance of such an evaluation has been illustrated by Capone *et al.* who compared the therapeutic concentrations of various non-selective and selective COX-2 inhibitors with the concentrations inhibiting by 80% COX-1 activity and 80% COX-2 activity (15).

Table 4. Discrepancies between the dose of different COX-inhibitors required per body weight in acute and chronic indications and the ED_{50} estimate obtained in pre-clinical models of pain. Dosing recommendations derived from a model-based approach would be based on exposures above IC_{80} values during the dosing interval. Data obtained from (3).

Drug	Therapeutic Dose (mg/kg/day)	Carrageenan model ED_{50} (mg/kg)	FCA model ED_{50} (mg/kg)
Diclofenac	2.1-2.9	1.6	0.99
Fenoprofen	11.4 - 45.7	58	15
Ketorolac	0.14 – 0.57	0.29	9.4
Naproxen	7.1 – 14.2	1.83	9.38
Rofecoxib	0.36 – 0.71	1.0	0.7

Interestingly, successful application of a pharmacokinetic-pharmacodynamic approach for the determination of dosage regimen has been reported in veterinary medicine in arthritis-induced febrile dogs. The authors show that dose selection of nimesulide based on PKPD relationships allowed appropriate control of pain and inflammation, but could not fully explain their findings since data on biomarkers were not collected during the experiment (16). Unfortunately, a similar reasoning still finds resistance in drug development and clinical medicine.

The impact of disease processes on drug effect was assessed by evaluating potency estimates after acute and chronic inflammation. The potency of naproxen was decreased under chronic inflammatory conditions. Yet, the selectivity based on the log IC_{80} COX-2/COX-1 ratio remained unaltered. Derived IC_{80} values for PGE_2 inhibition were less than 2-fold different for healthy and chronic inflamed rats (IC_{80} for PGE_2 inhibition is 8.5 versus 15.8 μg/ml for healthy and chronic inflamed rats, respectively. Derived IC_{80} values for TXB_2 inhibition are similar for healthy and

chronic inflamed rats (IC_{80} for TXB_2 inhibition is 3.2 versus 5.5 μg/ml for healthy and chronic inflamed rats, respectively). These differences cannot be assigned to pharmacokinetic factors and are likely to be solely driven by pathophysiological mechanisms described above.

Potential limitations. Unfortunately, limited data exists for chronic inflammation in the FCA model to allow further generalisation of these findings. Reduced potency in the adjuvant arthritis model was also observed for glucosamine (17). Our experiment does not allow complete exclusion of a methodological artifact of the FCA model itself as cause of the differences between acute and chronic inflammation, but we expect the latter to be the case. More data is required to discriminate how COX inhibitors affect inflammatory markers in different animal models of pain.

In conclusion, our results show the value of biomarkers instead of behavioural measures of pain as basis for the prediction of analgesic dose range in humans. These findings also seem to explain why different dosing regimens of COX inhibitors may be required for acute and chronic pain indications. A mechanism-based description of drug-disease interaction is essential to ensure appropriate evaluation of drug safety and efficacy in chronic inflammatory conditions. Given the rather unscientific way in which clinical findings and the underlying risk:benefit ratio for COX-2 inhibitors has been assessed, it is clear that without a radical change in the rationale for dose selection, patients may be prevented from getting access to valuable treatment options.

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