Chapter 11

Biomarkers of the anti-inflammatory effect and dose selection of COX inhibitors in early drug development: summary, conclusions and perspectives
INTRODUCTION AND OBJECTIVES OF THIS THESIS

The research described in thesis concerns the requirements for a model-based evaluation of the effects of COX inhibitors in inflammatory pain. The main objective is the development of a theoretical framework for the prediction, in a translational pharmacology context, of the anti-inflammatory effect of selective and non-selective COX inhibitors in humans using information from pre-clinical in vitro and in vivo experiments.

Few sensations are as disturbing to an individual as pain. Pain is accompanied by strong psychological and emotional components, as recognised by the International Association for the Study of Pain (IASP). This organisation has defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage". Hence, one must realise that the assessment of pain relief following drug treatment includes more than just the elements on the causal pathway of pain; it comprises contextual or circumstantial factors. The investigations described in this thesis plea for a different paradigm for dosing rationale, which relies upon a pathway analysis of the inflammatory cascade, dissociating drug action from the contextual factors associated with pain perception. Whilst clinical outcome should remain the ultimate goal of a therapeutic intervention, it is not acceptable to continue to disregard the mechanisms underlying the pharmacological response to COX inhibitors. The focus on target selectivity must be revisited to ensure incorporation of the recent advances in the understanding of the pathophysiology of inflammation.

Prediction of long term efficacy and safety has become one of the main challenges in the evaluation of non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of pain in chronic inflammatory conditions such as rheumatoid arthritis and osteoarthritis. Given the specificity in the mechanisms by which COX inhibitors modulate the inflammatory response, it is clear that target exposure may indisputably be associated with efficacy and safety, irrespective of the lack of a linear relationship between plasma concentrations and analgesic effect. Insight is needed into how time-dependency in transduction processes, including homeostatic feedback, is superimposed on the underlying exposure-effect relationship.

For the implementation of such a theoretical framework, one also needs to consider the role of experimental artefacts, such as differences in pharmacokinetics, biophase equilibration and transduction pathway, which undermine both face and construct validity of animal experiments (1). In a more clinical context, experimental artefacts also include design factors such as study design, inclusion criteria, endpoint selection, dosing regimen, sampling schedule and treatment duration. Hence, there are three distinct dimensions in the analysis of this problem: 1) integration of biological factors downstream in the inflammatory cascade under chronic disease conditions 2) identification of translational factors to account for differences between species, between health and disease, and between experimental conditions in vitro and in vivo and 3) impact of study design optimisation. These elements are sufficient to justify why empirical extrapolation of the efficacious doses in animal models of pain is a questionable approach.
From the aforementioned, it becomes evident that the lack of a direct correlation between drug exposure and analgesia does not mean that one cannot identify the dosing regimen required to achieve optimal treatment response. The question is whether an integrative tool exists that can embed all three dimensions of the analysis, unravelling markers or biomarkers of response and hence identifying drug exposure range to achieve optimal risk-benefit ratio. We strongly believe that mechanism-based modelling is essential to that purpose. Non-linear mixed effect models may not only contribute to the characterisation of the highly non-linear relationships between pharmacokinetics and pharmacodynamics, but also reveal translational factors and guide experimental design. It is anticipated that the modelling and simulation will become an essential step in the development of new analgesic drugs, as already occurred in other therapeutic indications such as in the development of anti-HIV drugs.

The overall goal of the research described in this thesis was to analyse the complex interrelationships between biomarker pharmacodynamics, pharmacokinetics and analgesic efficacy of COX inhibitors in chronic inflammatory conditions. Given the context of early drug development in which we operate, different aspects were taken into consideration, which regard the experimental procedures, analysis and interpretation of the data. Three main objectives were defined based on the use of PGE$_2$ and TXB$_2$ as biomarkers of pharmacology, namely, 1) to assess the relevance of PKPD relationships in vitro to predict the time course and extent of analgesic response in the target population in vivo, 2) to characterise the in vitro-in vivo correlations in rats and humans and 3) to demonstrate the value modelling and simulation to predict the optimal dose range in patients.

A specific objective of the investigations was the evaluation of the sensitivity of behavioural measures of inflammatory pain to the underlying PKPD relationships, as defined by biomarkers as intermediate endpoints. Special attention was paid to the variability and to the time course of response. In the various investigations we show that PGE$_2$ and TXB$_2$ inhibition can be used as biomarkers of COX activity, reflecting a process downstream on the causal pathway between target occupancy and analgesic response. In fact, our data also show that behavioural measures of analgesia fail to account for drug-induced changes in the inflammation cascade.

In this and the subsequent sections in this chapter, the results from the separate studies are briefly reviewed and final conclusions are drawn. Furthermore, a guideline and the perspectives for future research and development of novel analgesics are presented.

**PKPD CORRELATIONS AND BIOMARKERS IN THE DEVELOPMENT OF COX-2 INHIBITORS**

In chapter 3, a literature review was conducted to investigate the current understanding of the correlation between biomarkers of pharmacology and analgesic response in humans. To that purpose, the predictive value of in vitro-derived parameters for the estimation of the clinical dose of 22 selective and non-selective COX inhibitors was investigated. Systemic PGE$_2$ and TXB$_2$ production were measured using the human whole blood assay (hWBA). The plasma concentration at which
analgesia is achieved was correlated to in vitro estimates for COX-2 inhibition in humans. Initially, the IC\textsubscript{50} values for COX-2 inhibition, as obtained from the ex vivo whole blood assay, were selected as a link to analgesic concentrations. In this context, the role of protein binding was also considered; IC\textsubscript{50} values and therapeutic plasma concentrations were corrected for differences in free fraction. A correlation was found between IC\textsubscript{50} and the corresponding drug exposure for different COX inhibitors. However, the data also showed that more than 50% inhibition is required to achieve effective analgesia. For this reason, the model was reparameterised to obtain IC\textsubscript{80} estimates (i.e., drug concentration which produces 80% inhibition). Effective therapeutic concentrations were found to be directly correlated with IC\textsubscript{80}. From this correlation it is evident that COX inhibitors behave as antagonists, with at least 80% inhibition of COX-2 to yield an analgesic effect.

**PK-PD MODELLING OF THE BIOMARKER RESPONSE IN VITRO**

In this chapter, the main question was whether drug effects on biomarkers in vitro can be extrapolated in a strict quantitative manner from rats to humans. Based on the findings in chapter 2, IC\textsubscript{80} was selected as reference parameter in subsequent experiments. Pharmacokinetic-pharmacodynamic modelling of five different COX inhibitors was performed with respect to their effects on PGE\textsubscript{2} and TXB\textsubscript{2}, namely naproxen, diclofenac, ketorolac, rofecoxib and fenoprofen. These compounds were selected based on their in vitro selectivity for COX-1 and COX-2 as well as binding properties (2;3). In addition to the investigations involving blood sampling in rats and healthy subjects, data were also obtained directly from patients. In chapter 4, a PKPD model was proposed for the effects of naproxen on PGE\textsubscript{2} and TXB\textsubscript{2}, which at first suggests that parameter values (i.e., IC\textsubscript{80} values for PGE\textsubscript{2} and TXB\textsubscript{2}) in rats and healthy subjects are comparable or at least of the same order of magnitude. In chapters 6 and 10, the concept is expanded to include other compounds (diclofenac, rofecoxib, ketorolac and fenoprofen). For these compounds our results show that discrepancies may exist between species and extrapolation of data from animal to human may be of limited value. These discrepancies may be explained by differences in homeostatic regulation, biophase equilibration kinetics as well as by potential additional pharmacological activity at secondary targets, particularly for compounds which have known CNS-penetration. Therefore, data from these three chapters were further combined to enable a closer evaluation of the in vitro correlations across species. In figure 1, the in vitro IC\textsubscript{80} values in rats are correlated to data in humans for both PGE\textsubscript{2} and TXB\textsubscript{2}. In general, there is a tendency towards a direct one-to-one correlation between rat and human data for PGE\textsubscript{2} and TXB\textsubscript{2} inhibition. The assessment of such a correlation represented the first criterion for the construct validity supporting the use of animal data in the evaluation of novel COX inhibitors.

**IN VITRO-IN VIVO CORRELATIONS:TRANSLATIONAL PHARMACOLOGY**

A next step in our research programme was to investigate the in vitro-in vivo correlations for rat and human data. The in vivo effects of COX inhibitors on PGE\textsubscript{2} and TXB\textsubscript{2} must be characterised for that purpose. An important requirement is the possibility to accurately describe drug disposition in vivo.
General discussion and conclusions

COMPLEX PHARMACOKINETICS

Population pharmacokinetic models were developed to enable accurate assessment of the PKPD relationships of COX inhibitors. It is important to highlight that drug disposition and systemic exposure to COX inhibitors show complex, non-linear profiles. This is compounded by the limitations in blood sampling which require careful consideration of the experimental design. Since pharmacokinetic sampling may interfere with behavioural measures of analgesia, our experiments included sampling from the tail vein, which limits the number of samples that can be obtained from each single animal. This sampling method was chosen also because of the potential reduction in plasma albumin due to an acute phase reaction (4). Population pharmacokinetic modelling is a well established method to handle the analysis of sparse data as well as pooling of data from different experiments. In fact, additional pharmacokinetic experiments were performed to obtain a full exposure time profile of the different COX inhibitors. Drug exposure was investigated in healthy animals first. In a consecutive step, drug exposure was investigated under chronic inflammatory conditions.

The first difficulty to be dealt with was the non-linearity in the pharmacokinetics of naproxen in healthy and disease conditions (chapters 4 and 7). An increase in the volume of distribution was observed for naproxen in chronic inflammation, but these differences may not be clinically relevant compared to the impact of variability in pharmacodynamics. In fact, between-subject variability could not be estimated in chronic inflammation. The most important finding is that pharmacokinetics of naproxen was not altered, despite the progression of the inflammatory conditions, as assessed in the FCA model.

![Figure 1. Interspecies correlation for PGE\textsubscript{2} and TXB\textsubscript{2} inhibition in vitro. Compound names in black represent PGE\textsubscript{2} inhibition and grey names represent TXB\textsubscript{2} inhibition.](image-url)
A more complex pharmacokinetic profile was observed for rofecoxib and diclofenac (chapter 5). COX inhibitors like diclofenac and rofecoxib are subject to enterohepatic recirculation (EHC) in animals (5-10). EHC affects pharmacokinetic parameters such as bioavailability, clearance and terminal half-life. EHC-related changes in pharmacokinetics may consequently prolong pharmacodynamic effects or increase the risk of side effects, such as gastro-intestinal (GI) toxicity (11;12). Therefore, adequate prediction of the exposure time profile is required for the estimation of concentration-effect relationships for efficacy and safety endpoints.

The exposure to diclofenac and its metabolite 4-hydroxydiclofenac was described according to a model proposed by Waijma et al. (13), which includes a periodic transfer rate for the recycling process. Despite the absence of a gall bladder in rats, a bile excretion tract is present, which justifies the use of compartments for the recycling process. According to the model, the degree of recycling was independent of route of administration, study site and dose. A model with a periodic transfer rate was not suitable to describe enterophepatic circulation following administration of rofecoxib due to the large variability observed in systemic pharmacokinetics. Therefore, a three compartment model was developed, which included a conversion compartment with first-order rate and a lag-time to account for EHC. Regardless of the limited amount of data, the use of nonlinear mixed effects modelling enabled identification of inter-individual variability in the recycling process, as indicated by \( k_{\text{recycling}} \), the re-absorption rate constant. Furthermore, the inclusion of a mixture model allowed the identification of two distinct sub-populations in the concentration vs. time profiles of rofecoxib that accounted for a systematic pattern in the time course of concentrations in plasma, indicating different re-absorption rates in these two populations.

The pharmacokinetics of ketorolac was characterised by a three-compartment model after intravenous and intra-peritoneal administration (chapter 6), with body weight as a significant covariate of clearance. A one compartment model was required to characterise the pharmacokinetics of ketorolac after oral administration (chapter 8), which could be explained by differences in sampling schedule and rat strains for that route of administration.

Fenoprofen was added to the list of compounds aimed at our evaluation of the putative correlation between biomarkers in animals and humans. In contrast to the other COX inhibitors, fenoprofen is a racemic mixture that undergoes clinically significant in vivo chiral inversion of the R-enantiomer into the S-enantiomer, i.e., the active moiety. This process adds another level of complexity in the interpretation of pre-clinical findings, particularly if one takes into account that the metabolic clearance of the enantiomers differs significantly across species. The fenoprofen example clearly illustrates the flaw in the use of empirical extrapolation of animal data and highlights the importance of assessing exposure in conjunction with biomarkers of pharmacology prior to defining the dose rationale for the evaluation of COX inhibitors in humans. S-fenoprofen exposure in rats was described by a two compartment model with a combined zero and first order process for absorption. In this analysis the first order process describes the actual absorption of S-fenoprofen, whilst the zero
order process reflects the conversion of (-) R- into (+) S-fenoprofen. As the extent of conversion is greater in humans than in rats, the demand for a pharmacokinetic model that can describe the unidirectional biotransformation of R-fenoprofen more physiologically is higher in humans. Therefore, modelling of human pharmacokinetics in healthy subjects and in patients with systemic lupus erythematosus (SLE) included both enantiomers. A four compartment model with a transfer rate was capable of describing the conversion of (-) R- into (+)S-fenoprofen as well as the overall exposure profile in plasma.

![In vitro-in vivo correlation for drug effects on biomarkers in rats. Compound names in black represent PGE2 inhibition and grey names represent TXB2 inhibition.](image)

**Figure 2.** In vitro-in vivo correlation for drug effects on biomarkers in rats. Compound names in black represent PGE2 inhibition and grey names represent TXB2 inhibition.

**PK-PD MODELLING OF THE BIOMARKER RESPONSE IN VIVO**

In addition to the analysis of biomarkers *in vitro*, the pharmacokinetic-pharmacodynamic relationship of COX inhibitors was assessed *in vivo* for five different COX inhibitors, with varying degrees of selectivity. The effects of naproxen, diclofenac, ketorolac, rofecoxib and fenoprofen on PGE2 and TXB2 were assessed following systemic administration of these drugs at doses with putative analgesic effect in humans and in experimental models of inflammatory pain. Given the potential interference caused by sampling procedures via arterial cannulation, sparse blood samples were obtained from the tail vein. In conjunction with the pharmacokinetic models described above, non-linear mixed effect modelling was applied to derive full individual concentration-effect relationships. In principle, drug-induced effects on PGE2 result from suppression of cyclo-oxygenase activity. This chain of events could be characterised by an indirect response pharmacodynamic model. However, parameters identifiability for such structural model was not possible due to the high turnover rates involved in this cascade of events. Therefore, a direct inhibitory E\textsubscript{max} model was used to describe the concentration-effect relationship for the different COX inhibitors. The PKPD relationships of the inflammatory markers showed large variability over time. We cannot discard the role of noise from the bio-analytical methods, but believe that it can be explained to some extent by the circadian
variation in cyclo-oxygenase activity. As shown in chapter 4, clock time was introduced as a function of $I_{\text{max}}$ to account for the differences observed in the response profiles of experiments that started in the morning and in the afternoon.

In contrast to the in vitro correlation across species, the in vitro-in vivo correlation within species is less pronounced (figure 2). A prediction of drug potency or IC$_{80}$ in vivo in rats based on IC$_{80}$ estimates from in vitro experiments seems therefore difficult. For ketorolac, fenoprofen and rofecoxib, in vitro IC$_{80}$ values are lower than in vivo values, whereas for naproxen and diclofenac in vivo IC$_{80}$ values are higher. In humans, a close correlation was found between in vitro and in vivo IC$_{50}$ values (chapter 6), which strongly suggests the need to avoid the use of animal data for extrapolation purposes and prediction of the analgesic dose in humans.

An evaluation of the confounding effects of disease on the pharmacokinetic-pharmacodynamic relationships is illustrated by the use of naproxen in the FCA-model of inflammatory pain. Under chronic inflammatory conditions, it was shown that drug potency may vary over time, following the dynamics of the inflammatory response. In animals, parameter estimates for naproxen differed by only 2-fold, indicating reduction in the potency, which may have little or no clinical meaning. Model estimates suggest therefore that neither tolerance nor disease modifying effects occur during the course of treatment. More interesting was the considerable increase in the variability of PGE$_2$ and TXB$_2$ levels over time.

An evaluation of the impact of disease processes on the PKPD relationships and consequently on the interpretation of in vitro as well as in vivo data was further explored using fenoprofen as model drug. In addition to experiments in rats, fenoprofen was studied in healthy subjects and SLE patients. Interestingly, fenoprofen was 2-fold more potent in SLE patients than in healthy subjects with regard to TXB$_2$ inhibition, whereas no differences were observed between groups with regard to PGE$_2$ inhibition. In vivo IC$_{80}$ estimates in rats also correlated well with data in healthy subjects. Simulations were conducted to investigate whether data in humans or in rats would best predict the dose range which is required for analgesia in SLE patients. Our results show that in vitro human data best describes biomarker effect in patients.

A subsequent step in the analysis of PGE$_2$ inhibition in vivo is to assess PGE$_2$ levels without making use of LPS stimulation, as a control or reference for the changes observed under the experimental conditions. Moreover, it would be useful to link COX-2 activity to PGE$_2$ levels. This would provide information on the enzyme capacity under basal conditions and during inflammatory response. The magnitude of variability in response measures (biomarkers) cannot be explained solely by experimental noise. Our data suggests that variability in response is due to differences in the biological substrate, i.e. in inflammatory status.

**EXTRAPOLATION OF THE BIOMARKER RESPONSE TO THE ANALGESIC EFFECTS**

Ideally, the pharmacodynamic responses assessed in vivo in pre-clinical models should be biomarkers
or surrogates of drug effects in patients. However, their relevance or predictive value remains a key issue in clinical pharmacology. Despite major efforts in achieving high degree of COX selectivity during drug design, pre-clinical screening of compounds is still based on qualitative rather than quantitative measures of drug effect in vivo, most of which bear no correlation with the underlying mechanisms of action (14). Another important issue is that the selected pharmacodynamic endpoint should be sensitive in discriminating between compounds with the same mechanism of action. In chapter 8, the time course of inflammation was assessed by weight bearing capacity, dynamic plantar test and paw pressure threshold, as commonly used in pre-clinical development programs. The use of these behavioural tests is deemed an appropriate measure of drug effect on static allodynia, dynamic allodynia and hyperalgesia, respectively.

Hyperalgesia was found to persist over four weeks after FCA injection. In contrast, allodynia, which was assessed by weight bearing capacity and dynamic plantar test, waned over time. Remarkable was the observation that none of the COX inhibitors under evaluation showed an effect on static alldynia, as measured by the dynamic plantar test, whilst their effect on weight-bearing capacity was rather variable, without any correlation between the time course of response and pharmacokinetics. On the other hand, all compounds showed a significant effect on hyperalgesia, with clear dose-dependent effect after administration of increasing doses of naproxen and rofecoxib. In addition, no differences were observed in the magnitude of response after single administration seven days after FCA-inoculation or after twice-daily dosing until 21 days post-inoculation. These results strongly suggest that COX inhibitors do not have disease modifying properties, nor lead to the development of tolerance.

Thus, behavioural endpoints associated with static and dynamic allodynia are insensitive to COX inhibition or lack sensitivity to varying degrees of COX inhibition. Furthermore, the results for rofecoxib suggest differential systemic effects between selective and non-selective COX-2 inhibitors, raising questions about the relevance of drug distribution (biophase equilibration) as well as the role of central inhibition of COX-2, compared to drug action at the site of inflammation. Our results support the use of paw pressure threshold in the FCA model as the only suitable behavioural measure in the screening of COX inhibitors. Changes in paw pressure threshold seem to reflect the anti-hyperalgesic properties of these compounds with enough sensitivity to enable the estimation of a dose-exposure-response curve. However, it is clear that allometric scaling of behavioural responses based on weight differences across species will not provide accurate estimates of a clinically effective and safe dose range. The rationale for dose selection in humans must also consider the extent and duration of drug effect on both biomarkers PGE2 and TXB2. In fact, a mechanism-based modelling approach is required to further characterise the relationship between pharmacokinetics, biomarker inhibition and analgesic effect.

In chapter 9, the relationship between pharmacokinetics, biomarkers and analgesic response was analysed with different population PKPD models. High variability was observed for the biomarker response and data showed no unique concentration-effect relationship. Analysis of the biomarker-analgesic effect relationship was performed using two different approaches. First, a logistic regression
was used to characterise the changes in pain threshold. Subsequently, a mechanism-based model was developed to analyse the data as a continuous variable. The paw pressure threshold was dichotomised, in a first attempt to filter the noise in the data. Paw pressure values were calculated as percentage relative to the control value (non-inflamed paw) at the time of measurement. A response was defined if the difference between inflamed and control was less than 20%. This analysis revealed the presence of time dependencies between biomarker and response, which render the PGE\(_2\) levels a predictor of the analgesic response only in the first 3 hours after dosing. Biomarker levels at a later time were unable to accurately predict analgesia. Interestingly, the potency of rofecoxib was two-fold lower compared to the other COX inhibitors (EC\(_{50}\) is 41.7 ng/ml for rofecoxib versus 19.3 ng/ml for naproxen, diclofenac and ketorolac). Our results also revealed that TXB\(_2\) levels do not correlate with analgesia, implying that COX-1 activity varies considerably during chronic inflammation.

A mechanism-based model for the relationship between drug exposure, biomarker and analgesia was developed under the assumption of indirect responses with a feedback loop, capturing the dynamics of pain signalling, pain perception and counter-regulatory mechanisms observed in chronic inflammation. The inhibition of PGE\(_2\) by the different COX inhibitors was correlated with drug exposure. However, the analgesic response was short-lasting compared to the inhibition of the biomarker for three out of five compounds. It is hypothesized that long-term suppression of COX triggers the formation of mediators responsible for sustained pain signalling which offset the effects of PGE\(_2\) inhibition, such as leukotrienes (LTs). In fact, evidence from published literature suggests that LTs, which are synthesised through the lipo-oxygenase (LOX) pathway, are involved in the production and maintenance of inflammation. Therefore, model parameterisation included a putative counter-regulatory loop in which PGE\(_2\) levels triggers the production of algesic mediators. An interesting finding was that parameter estimates describing counter-regulation were significant for naproxen and ketorolac, whereas these were negligible for rofecoxib. In contrast, diclofenac-induced analgesia outlasted PGE\(_2\) inhibition. Given the range of concentrations used for each compound, these findings seem to suggest that COX selectivity may determine the extent effect, but not its duration. These discrepancies may also point out to the contribution of additional targets or modes of action of these two compounds. In fact, published data have shown that these drugs also have specific effects on the spinal cord and central nervous system, which may be COX independent.

Another possible explanation for the differences in response could originate from different pathways exhibiting both pro-and anti-inflammatory activities (depending on organs and disease stage) (15).

In conclusion, we have shown that a pathway analysis is required to describe the correlation between drug exposure, biomarker and analgesic effects. Our results reveal that currently accepted animal models may lack construct validity, in that COX selectivity and downstream effects upon COX inhibition may not reflect drug action in humans. It is also clear that COX selectivity may be a determinant for the extent of PGE\(_2\) inhibition, but does not govern the duration of the effect, as demonstrated for compounds with varying degrees of selectivity. The sole use of behavioural measures in pain models overlooks the differences in the pharmacology of COX inhibitors that ultimately drive response and adverse events. The use of biomarkers in humans is therefore
recommended as basis for the selection of the clinical dose range. Whilst focus is given to efficacy when defining a dose range, we have shown that the use of modelling and simulation enables an integrated evaluation of multiple endpoints, providing a stronger rationale for dose selection and better estimation of the risk-benefit ratio. Our approach can be particularly relevant for a quantitative analysis of treatment response following combined drug therapy.

**FUTURE PERSPECTIVES**

COX-3

It has been more than 36 years since Sir John Vane first reported that the pharmacological actions of aspirin-like drugs could be explained by their ability to inhibit cyclo-oxygenase (COX). Since then, a second isoform of COX, named COX-2, has been discovered and highly selective inhibitors of this isoform have been marketed. Most recently in 2002, a splice variant of COX-1 mRNA retaining intron 1 has been described, which was given the names COX-3, COX-1b or COX-1v. The discovery of COX-3 as an acetaminophen-sensitive isoform seemed to solve the mystery of the mechanism of action of acetaminophen and attracted considerable attention, and several commentaries were published quickly in prominent scientific journals. This discovery in canines was generalised to rodents and humans without critical evaluation of the experimental data. Its low expression level and the available kinetic data indicate however that this selective interaction is unlikely to be clinically relevant (16). Although several years have passed since the original publication, there is no follow-up study that would confirm the COX activity of the canine COX-3 and its sensitivity to acetaminophen. In humans and rodents an acetaminophen sensitive COX-3 protein is not expressed because the retention of intron-1 adds 94 and 98 nucleotides to the COX-3 mRNA structure respectively. Since the genetic code is a triplicate code (3 nucleotides to form one amino acid), the retention of the intron in both species results in a frame shift in the RNA message and the production of a truncated protein with a completely different amino acid sequence than COX-1 or COX-2 lacking acetaminophen sensitivity. The biological function of the COX-3 protein is currently unknown. COX-3 proteins from dogs, humans, and rodents do not show substantial homology, which would be expected from a protein with physiological function. Therefore, a specific therapeutic role for COX-3 in the development of drugs for chronic inflammatory conditions can be regarded as improbable.

**NEW TECHNOLOGIES**

Understanding of drug activity at the site of action may be necessary to further understanding the dynamics of inflammatory response. New technologies could provide more information on the role of COX-2 inhibition throughout the body. For example positron emission tomography (PET), which is a non-invasive imaging technique, can be used to detect biochemical and biological processes *in vivo* at sub-nanomolar levels and may therefore be valuable in chronic inflammatory conditions (17).
Chapter 11

INTERSPECIES EXTRAPOLATION

An important question at the beginning of this research project was whether biomarker response *in vitro* or *in vivo* in pre-clinical species can be used to predict drug exposure levels that are required for appropriate analgesic response in humans. Animal models of pain have been considered for many years as an essential step in the evaluation of analgesic drugs aimed at the treatment of chronic inflammatory conditions. However, as shown throughout this thesis, the lack of a clear concentration-effect relationship makes the decision about the first dose in humans a critical issue. Interspecies scaling for the analgesic response in humans has been performed predominantly on a dose per kg body weight basis. A less empirical approach for interspecies extrapolation is required to accurately define the dosing rationale in humans. Scaling should be based on the relationship between drug exposure and inflammatory response. An important component of this approach is the characterisation of the relevant pathways associated with biomarker response. Based on our results, pharmacokinetic-pharmacodynamic modelling of *in vitro* data can be used to assess such relationships and scale dose requirements from animals to humans. In fact, we have also shown that the characterisation of PKPD relationships can assist with extrapolation of data from healthy status to disease conditions.

Another challenge in translational research is to establish the link between biomarkers of the anti-inflammatory effect *in vivo* and the analgesia in patients. Part of the difficulties in the evaluation of the correlation between biomarkers and pain intensity or pain relief in humans includes the categorical nature of clinical scales and the high nonlinearity between pain signalling and pain perception.

An other aspect in the translational research concerns the high variability in the analgesic response is observed in patients. The variability observed in the PGE$_2$ response can be considered "small" compared to the variability in the clinical endpoint. This implies that not all variability in patients can solely be explained by the variability caused by the antagonist binding to the target COX and the inhibition of PGE$_2$.

One should characterise the pharmacological drug action in terms of drug specific properties and system specific properties. A key element of mechanism-based PKPD modeling is the explicit distinction between drug-specific and biological system-specific parameters (18). Drug-specific parameters describe the interaction between the drug and the biological system in terms of target affinity and target activation, whereas system-specific parameters describe the functioning of the biological system. The distinction between drug-specific and biological system-specific parameters is crucial for predicting drug effects in humans accurately. Variability in the extent and duration of the clinical response in patients could come from system-related differences such as differences in feedback mechanisms, differences in cascading events after binding of COX inhibitors to the target COX. Variability in PGE$_2$ inhibition between patients can only partly be explained by drug-specific differences, more likely are the differences in system-specific parameters. Important other factors include gender, age, genetic polymorphisms in different enzymes, differences in disease status, co-medication, feedback mechanisms and second messengers (19-21). Lee et al showed that
polymorphism in COX-2 partly explains variability in visual analog scale (VAS) scores of COX inhibitors (22). The importance of a model-based approach to characterise this link will be discussed later in this chapter.

It is evident that a similar challenge exists for the extrapolation and prediction of long term safety based on short term drug effects. Given the dynamics of inflammatory response and the physiological roles of COX-2 and prostacyclins, accurate predictions of drug-induced adverse events cannot be made unless pathophysiological processes and disease progression are incorporated into the analysis of the data. To that purpose, other markers of the inflammatory cascade may need to be investigated in conjunction with PGE2 and TXB2, so that different aspects of drug action can be explored over time. The implications of long term exposure to treatment can then be drawn not only from actual observations in a clinical trial or animal experiment, but also from a scenario analysis.

CARDIOVASCULAR SAFETY

The withdrawal of rofecoxib from the market in 2004 (Vioxx®, Merck & Co, Inc.) and six months later of valdecoxib (Bextra®, Pfizer) because of the increased risk of myocardial infarction and stroke associated with chronic treatment has caused scepticism about the future of COX-2 inhibitors. The other selective COX-2 inhibitor available at that time was celecoxib (Celebrex®, Pfizer), which remained on the market. Celecoxib may be less harmful than rofecoxib and valdecoxib - possibly because it is an order-of-magnitude less selective for COX-2 than its cousins - so the FDA has allowed it to be marketed, with clear warnings of its cardiac risks (23). Subsequently, lumiracoxib (Prexige®, Novartis) was rejected by the FDA in September 2007, because of serious liver side-effects. Hence, from the above it is clear that the safety of COX-2 inhibitors has become more relevant than its analgesic response. An explanation for these findings can be found in the underlying mechanisms of action. There is evidence showing that COX-2 also catalyses the production of prostacyclin (PGI2). PGI2 supposedly protects the heart by reducing blood clotting and by relaxation of blood vessels. Consequently, suppression of this hormone is thought to be associated with heart attacks and strokes, which would make this a class effect, rather than a drug specific adverse event.

The problem of the increased cardiovascular risk with the chronic treatment of selective COX-2 inhibitors demands separation of benefit from risk in a more quantitative, mechanism-based manner. Appropriate dose selection plays an essential role in this evaluation. Given the intricacy in the inflammatory response, it is clear that continuous suppression of PGE2 by the selective COX-2 inhibition should be avoided. To illustrate how dose level and dosing regimen determine the extent and duration of PGE2 inhibition, we have simulated lumiracoxib plasma concentrations with the corresponding PGE2 levels (figure 3) for multiple scenarios, based on a four week period of chronic dosing. Recommended doses are 100 mg QD for osteoarthritis and 200 mg QD for primary dysmenorrhea. The recommended dose for acute pain (400 mg QD, maximum of 5 days treatment) was not included in this simulation. In contrast to once daily dosing, twice daily dosing (50 and 100 mg BID) was also simulated as a possible dosing regimen and compared the inhibition profiles for
PGE$_2$ obtained for once daily dosing. Lumiracoxib plasma concentrations were simulated based on an article by Scott et al. (24). The potency for lumiracoxib (IC$_{50}$ 117.4 µg/L) was based on the human in vitro whole blood assay (25).

Once daily dosing for lumiracoxib showed a continuous PGE$_2$ inhibition, reaching more than 80% inhibition, even at the lowest recommended dose of 100 mg QD. On the other hand, twice daily dosing resulted in a lower degree of inhibition and less fluctuation in biomarker levels during dosing interval. Based on these preliminary simulations, it appears that once daily doses may be sufficient to retain basal activity of cyclo-oxygenase. As can be seen from this example, this type of inference offers an opportunity to evaluate the contribution of the underlying pharmacological mechanism to the event of interest. Of course, a thorough analysis must comprise other factors contributing to cardiovascular risk, such as co-morbidities, age, etc. In fact, the value of such a model-based approach has been recognised by the EMEA as an important tool in the characterisation of drug response.

Figure 3. Simulation of the effects of lumiracoxib on biomarkers. Pharmacokinetics (shown on the left) was simulated from data by Scott et al. In vitro potency in the human whole blood assay (IC$_{50}$ 0.4 µmol/L) was used for the analysis of drug effects on PGE$_2$ (shown on the right). Four different scenarios (BID and QD) were simulated based on the therapeutic doses in OA (100 mg QD) and primary dysmenorrhea (200 mg QD).

A scenario analysis offers a different perspective to the evaluation of efficacy and safety, which reveals elements which may not be extracted from experimental observation, often because of design limitations, but which are essential to explain short and long term effects of drugs with known mechanism of action. In this sense, it is paramount to shift away from the current empirical, descriptive nature of safety data analysis. Methods must be developed which incorporate the pathways underlying inflammatory response and relevant disease processes, so that drug-specific and biological system-specific parameters can be assessed and differentiation can be made between compounds, dose levels and dosing regimens.
GASTROINTESTINAL SAFETY
The association between NSAIDs and gastrointestinal erosions and ulcers is well established. In fact, gastro-intestinal complications are recognised as the most prevalent and severe cause of drug toxicity in the USA (26). NSAID gastrointestinal damage is mediated through several mechanisms that compromise mucosal integrity, including the inhibition of platelets even at low dosage, which gives rise to bleeding and hemorrhagic events (27).

Data from large-scale clinical trials have confirmed that the COX-2 inhibitors are associated with substantial reductions in gastrointestinal risk in the majority of patients who do not receive aspirin. Clinical studies suggest that the risk for gastrointestinal adverse events by COX-2 inhibitors is approximately equivalent to the use of proton pump inhibitor (PPI) therapy in conjunction with traditional NSAIDs. As a matter of fact, the withdrawal of selective COX-2 inhibitors from the market has led many physicians to consider the use of traditional NSAIDs in combination with a PPI to reduce the gastrointestinal side effects of NSAIDs. However, the gastro-protective benefit of COX-2 inhibitors is partially or, in some patients, totally offset if aspirin is used for cardiovascular prophylaxis (28). Reducing the risk for gastrointestinal complications requires therefore a thorough understanding of potential complications and underlying predisposing risk factors, which is a particularly important consideration in light of the fact that many patients develop complications without antecedent warning signs or symptoms. Numerous risk factors are known to increase substantially an individual's risk for NSAID-induced gastrointestinal events (29). These include a history of ulcer, presence of Helicobacter pylori infection, use of more than one NSAID (including aspirin), use of high-dose NSAIDs, concurrent anticoagulant or corticosteroid use, a serious underlying disease, and age greater than 75 years. The severity of RA may also be directly related to an increased risk for gastrointestinal events (30).

From the abovementioned, it becomes evident that selecting a combination of therapies that provides relief from arthritis related symptoms, minimises cardiovascular risk, and preserves the gastrointestinal mucosa is a complex challenge. Factors to consider include the interference of certain NSAIDs, such as ibuprofen, with the antiplatelet effects of aspirin; direct effects of non-selective NSAIDs and COX-2 inhibitors on fluid retention and blood pressure; emerging data about cardiovascular risks associated with these drugs; differences in the adverse gastrointestinal event rates among NSAIDs and COX-2 inhibitors; and the feasibility of co-therapy with gastro-protective agents (30).

Selectivity does play a role in terms of the pharmacological properties of a compound, but as such is not sufficient to allow predictions about drug efficacy and safety. The complex interaction between the various mediators of inflammation during chronic disease conditions cannot be evaluated by mere description of the affinity of drug and its primary target. The integrated pathway analysis proposed in this thesis, in which disease components are evaluated in conjunction with PKPD modelling could play an important role in the assessment of the appropriate dose for combination therapies, as well as and provide evidence for rational drug combinations. Furthermore, this type of analysis would provide a proxy for the evaluation of long term safety and potential for class effects.
MECHANISTIC MODELS

What Celsus defined in De Medicina as 'rubor, calor, dolor, tumult' (redness, heat, pain and swelling) is an intellectual challenge in signal transduction and systems biology, as well as a multibillion dollar market for the pharmaceutical industry. The area of PKPD modelling is evolving towards a more mechanistic, biological systems approach. Despite the increase in the complexity of such models, parameterisation of the steps involved in the transduction process can be a valuable tool in the evaluation of the interaction between drug and biological system. Moreover, it clarifies the cause of time dependencies in biological processes.

Inflammation and inflammatory pain in particular may be considered in terms of its checkpoints, where higher-order signals drive each commitment to escalate, go signals trigger stop signals, and molecules responsible for mediating the inflammatory response also suppress it, depending on timing and context (e.g. PGE2, TNF-a, IFN-g, TGF-b) (31). The non-inflammatory state does not arise passively from an absence of inflammatory stimuli; rather, maintenance of health requires the positive actions of specific homeostatic factors to suppress reactions to potentially inflammatory stimuli that do not warrant a full response. These interactions are exactly within the realm of a biological systems analysis.

The central dogma of biological systems analysis states that it is system dynamics and organising principles of complex phenomena that give rise to the functioning and function of cells. Since cell functional activity, including differentiation and apoptosis, are time dependent processes, they can only be treated as dynamic systems. In this context, mathematical modelling is an essential feature contributing to the understanding of causal interactions in cells. Causation is the explanation of change but while changes occur in the realm of matter, the credo of dynamic systems theory is that causation is modelled as a relation between changes of states. Thinking of a pathway as a network or cascade of events, the state of the system is defined by the levels of all components, including drug concentrations. The (dynamic) behaviour of a system is thus encoded in the temporal evolution of its state. For anything to change in time and/or space, differential equations, automata, and other symbolic representations offer themselves as a natural language for encoding observations made in experiments.

Moreover, dynamic behaviour implicitly indicates the existence of a goal or objective, which is evident in the case of inflammatory pain. In fact, the claim that dynamic interactions of system variables give rise to cell functions means that adaptation, maintenance and optimisation principles are required. In order to induce changes towards that goal or objective, information must be fed back so that the consequences of change can be accounted for. Feedback loops imply a "before" and an "after", which cannot be described otherwise. A trend in the analysis of intra- and intercellular dynamics is the study of characteristic behaviour, including oscillations and switching, which can be very pertinent to pain signalling in chronic inflammatory conditions. One can distinguish between two key functions underlying such regulatory mechanisms. In the first case, it involves situations in which the system is sensitive to external inputs and has the ability to make changes, as required. On
the other hand, one can refer to homeostasis or regulation as the maintenance of a desirable state (robustness), preventing changes or perturbations. Here one should distinguish two forms of robustness in a control system. The first is robustness against external disturbances (disturbance regulation). In a pathway, a disturbance might be caused by unwanted cross-talk among pathways or undesirable signals from one or a few neighbouring cells. The second form of robustness is one which tolerates parameter changes in a system, without significantly changing the system performance. The identification of dynamic motifs and the development of concepts to investigate the regulation, control and coordination of inflammatory cascades are therefore crucial to the advancement of treatment of chronic pain.

We anticipate that the major challenges in dynamic pathway modelling for the coming years will include characterisation of model structure and system identification (i.e., to determine values for model parameters using experimental data or simulation studies) as well as control analysis to predict the consequence of changes to a pathway; in particular modifications to parameters, cross-talk and the introduction and removal of feedback loops. This exercise will be essential to the assessment of long term effects of single drugs as well as combined drug therapy.

GUIDELINE FOR DRUG DEVELOPMENT OF ANALGESICS
The delicate balance between efficacy and safety for COX inhibitors as first choice drugs in the treatment of chronic inflammatory pain must be investigated in view of the underlying pathways, rather than by judgment of isolated drug properties such as selectivity and potency. Mechanism-based modelling of drug effect on biomarkers of inflammatory response and clinical trial simulations is required for the evaluation of the appropriate dose range and dosing regimen of selective COX-2 inhibitors in the future. Both the scientific community and regulatory authorities must recognise the importance of assessing drug effects on biomarkers for optimisation of the therapeutic use of these compounds and accurate interpretation of the findings in early drug development (32).

Based on the work presented in this thesis, animal models such as the FCA model hamper the predictability of the pain response to humans as indicated by the complex biomarker-analgesic response relationship in rats. In an analogy to René Magritte's pipe, it can be said for animal models of pain that ceci n'est pas une response inflammatoire, ceci n'est pas un modèle.
In vitro biomarkers can and should be used to predict the optimal dose range in patients as well as the consequences of inappropriate compliance. They provide valuable information about the underlying pharmacological effects, which are overlooked by behavioural measures of analgesia. In conjunction with modelling and simulation, the availability of such information is crucial for differentiating novel compounds in terms of efficacy and most importantly in understanding their safety.
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