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

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Chapter 3

Scope and intent of investigation

I GENERAL OBJECTIVES

The mechanism by which cyclo-oxygenase (COX) inhibitors exert their analgesic effect is well established. However, given the complexity in nociceptive mechanisms and the nature of measures of analgesia *in vivo*, often no direct correlation is observed between drug concentrations in plasma and treatment response in chronic inflammatory conditions. As indicated in the introduction, this represents a major problem in the development of COX inhibitors. Specifically it is difficult to predict the appropriate dosing regimen for the treatment of chronic inflammatory pain, based upon information from pre-clinical studies and eventually early clinical studies. Moreover, the use of behavioural endpoints or clinical symptoms by itself prevents the characterisation of drug activity on the primary and secondary targets, precluding an assessment of the potential for long term adverse events. The factors that determine the treatment response in inflammatory pain must be taken into account in order to make predictions about the time course of the analgesic effect and consequently to select the appropriate dosing regimen.

Based on the aforementioned, the identification of specific biomarkers to explain variability in pain response in chronic inflammatory diseases may be a essential factor for the selection of the dose range for COX inhibitors during clinical development, The use of a biomarker can provide a link between pharmacokinetics (PK) and drug effect as well as offer a proxy for safety evaluation. In fact, a number of mediators on the cyclo-oxygenase pathway could be used as intermediates between PK and analgesia. In conjunction with a model-based approach (non-linear mixed effect modelling), the relationship between biological marker, pain measurement and safety can then be characterised. Primary candidates for such a role are prostaglandins and thromboxanes.

The main objective of the research described in this thesis is to demonstrate the relevance of biomarkers on the selection of the dose range of COX inhibitors for effective analgesic and anti-inflammatory response, as opposed to the focus on behavioural measures of pain and inflammation advocated by the current paradigm for the development of non-steroidal anti-inflammatory drugs (NSAIDs). To this end, the relationship between drug concentration and the corresponding inhibition of prostaglandin E2 (PGE_2) and thromboxane B2 (TXB_2) was investigated for a range of COX inhibitors with varying degrees of selectivity, and hence with differential effects on the selected biomarkers. Thanks to the use of a mechanism-based approach, attention is also given to translational pharmacology in drug development. We evaluate whether 1) estimates of drug action *in vitro* are predictive of the effect *in vivo*, 2) animal data *in vivo* reflect drug effect on biomarkers in humans and 3) whether inflammatory conditions modify the extent of drug effect as compared to healthy conditions. A recommendation and guideline for best practices in the development of COX inhibitors is anticipated from this analysis.

A summary of the current research methodologies is provided in **chapter 2** by a comprehensive review of the literature on COX inhibitors. We confront various approaches and experimental procedures and challenge the meaning of the estimates obtained for drug potency, efficacy and

selectivity and their relevance for treatment response *in vivo*. In addition, we explore the consequences of COX-2 selectivity by evaluating how *in vitro* PGE_2 inhibition in the human whole blood assay (hWBA) correlates with therapeutic plasma concentrations at which analgesia is achieved. Throughout this thesis we will refer to *in vivo* conditions whenever a dose is administered and blood samples are collected for drug and/or biomaker quantification. In contrast, *in vitro* will be used to describe drug effect whenever COX inhibitors are added to a blood sample at a known concentration or concentration range. For the sake of clarity, we will not make reference to the *ex vivo* experimental procedures commonly used in literature publications on the whole blood assay. The review is concluded with a critical discussion of the importance of a model-based approach to integrate the various factors that ultimately contributing to drug response and explain variability in the clinic, such as physicochemical properties, pharmacokinetics, pathophysiology and disease progression.

II PHARMACOKINETIC-PHARMACODYNAMIC (PKPD) MODELLING OF BIOMARKERS

Initially, four COX inhibitors were selected as model drugs for the assessment of the pharmacokinetic-pharmacodynamic relationships *in vitro* and *in vivo*. The mechanism of binding and selectivity for COX-1 and COX-2 were used as criteria for selection of the compounds. Naproxen and diclofenac were selected for their rapid and time-dependent non-selective binding properties, whilst ketorolac and rofecoxib were chosen for their selectivity to COX-1 and COX-2, respectively.

In **chapter 4**, we assess the PKPD relationship of naproxen *in vitro* and *in vivo*. Assuming no time-dependencies in binding and consequently reversible, immediate pharmacological effect upon inhibition of cyclo-oxygenase, a sigmoidal E_{max} model is used to characterise the inhibition of LPS-induced PGE_2 as well as the anti-platelet effect following suppression TXB_2. Inferences are made about the effective dose and the corresponding risk-benefit ratio in humans based on drug effect on biomarkers, rather than behavioural measures of pain. In addition, we explore how to best account for between-species differences and evaluate the requirements to scale pharmacodynamic parameters from rats to humans.

Since time-dependent binding can lead to a disconnection between drug effect and pharmacokinetics in plasma, consideration was given to various aspects of drug disposition that could alter the duration and/or time course of anti-inflammatory response. Of particular interest is the enterohepatic circulation (EHC) observed for rofecoxib and diclofenac. The aim of **chapter 5** was therefore to compare the performance of different pharmacokinetic models of EHC for diclofenac and rofecoxib in rats. Pharmacokinetic model parameters were subsequently used in conjunction with an Emax model to simulate drug-induced PGE_2 inhibition and thereby evaluate the role of EHC on pharmacodynamics.

In **chapter 6**, we investigate the translational aspects of pharmacology of COX inhibitors by comparing their inhibitory effect on PGE_2 and TXB_2 *in vitro* in rat and human blood and *in vivo* in

rat and humans. In addition to compounds initially selected for the investigation of the relevance of *in vitro* selectivity for COX-2 (rofecoxib) and COX 1 (ketorolac) as well as of time-dependency in binding (naproxen and diclofenac), various other drugs are included from published literature. Since one of our major concerns is the need for a sound rationale for dose selection in early drug development, different (re-)parameterisations of drug effect were considered. Instead of relying on IC_{50} as potency estimates, we propose the use of IC80 as allometric factor between species. Given the differences between compounds in the slope of the concentration-effect curves, it is hypothetised that IC_{80} may reflect more closely the pharmacological properties of enzyme inhibitors *in vivo*.

On the other hand, the use of COX inhibitors in chronic diseases has raised important questions about on long term efficacy and safety. Hence, in **chapter 7** we assess the impact of disease processes on biomarker response in an animal model of chronic inflammation for naproxen as compared to healthy conditions. From a translational pharmacology perspective, it is evaluated whether dose adjustment is required over the course of treatment and whether a translational factor can be found between short and long term effects. In addition, we also ascertain whether biomarker data predicts drug response *in vivo* during chronic inflammatory conditions, enabling replacement of animal models of pain and other pre-clinical experiments *in vivo*.

III PHARMACOKINETIC-PHARMACODYNAMIC MODELLING OF ANALGESIA

To establish the relevance of biomarkers in the evaluation of the analgesic response to COX inhibitors, drug effects must be assessed under disease conditions. In this context, the choice of which animal model to use is essential for the correct extrapolation to the clinic. Thus far, little attention has been paid to the possible discrepancy between behavioural measures of analgesia in pre-clinical models of pain and pain response associated with chronic inflammatory conditions in humans. To our knowledge no investigation has demonstrated in a quantitative manner how pharmacology translates into analgesia in these models, nor whether different behavioural endpoints are equally sensitive to drug effects. Specifically, one should understand the underlying mechanisms of the pain response as well as the cause of differences in pain perception to predict analgesia and the corresponding variability in response within a patient population. Moreover, one needs to elucidate which behavioural endpoint is most sensitive to the pharmacological effects of COX inhibitors, so that correlations can be made between drug action in animals and humans.

In **chapter 8**, we use the Freund's complete adjuvant (FCA) model of inflammatory pain to compare the analgesic effects of four COX inhibitors (diclofenac, naproxen, ketorolac and rofecoxib) in rats. Moreover, we investigate the correlation between the degree of selectivity for COX-1 and COX-2 and the extent of analgesia, as assessed by different behavioural endpoints, namely mechanical hyperalgesia, weight-bearing capacity and static allodynia.
Another scarcely understood and interesting aspect of treatment response in chronic inflammatory conditions involves the assessment of treatment-induced disease modifying effects, which is quite pertinent to the various therapeutic indications of COX inhibitors. To address this question and

establish whether the timing of intervention matters, rofecoxib was used as a model drug in an experimental design including pre-emptive versus post-emptive treatment groups.

Based on the evaluation of the sensitivity of different behavioural measures of hyperalgesia and allodynia, in **chapter 9** a first endeavour is made to establish the relationship between drug exposure, biomarkers and analgesic effect, as assessed by mechanical hyperalgesia in the FCA model in rats. Using modelling and simulation techniques, we attempt to explain the discrepancies between the time course of biomarkers and hyperalgesia. The presence of nonlinearity and feedback mechanisms in the processes underlying transduction and pain response confirm our initial proposition for a mechanism-based approach to accurately predict the dose range and dosing regimen of COX inhibitors in humans.

IV CLINICAL EXTRAPOLATION AND INTER-SPECIES SCALING

In **chapter 10**, the application of biomarkers in drug development is illustrated by the evaluation of potentially effective doses of fenoprofen as an anti-inflammatory agent in systemic lupus erythematosus. Using an integrated analysis of the concentration-effect relationships of fenoprofen on PGE_2 and TXB_2 we demonstrate how dosing regimen optimisation can be achieved in this patient population and prospectively during clinical development. We also discuss how differences in the PKPD relationships *in vitro* and *in vivo* as well as across species may lead to inaccurate dose selection. This exercise highlights the importance of biomarkers to enable interspecies scaling and appropriate interpretation of *in vivo* measures of pain, which are determined not only by the target associated with the pharmacological intervention but result from a dynamic system in which the time course and magnitude of response are modulated by feedback mechanisms and interactions.

V CONCLUSIONS AND PERSPECTIVES

The results of the investigations described in this thesis are reviewed and discussed in **chapter 11**. In addition to a comprehensive discussion of the implications of the complexity in the mechanisms underlying drug effects in inflammatory pain, focus is given to the role of translational pharmacology research in drug development. We conclude by emphasising how disease analysis, modelling and simulation can facilitate the integration of biomarkers in the rationale for dose selection in clinical studies. Such integration is an essential step towards accurate prediction of drug efficacy and safety in chronic inflammatory conditions. Drug development in the field of analgesics should be based on biomarkers rather than pre-clinical animal models. *In vitro* biomarker data in humans best predicts the *in vivo* analgesic effects in humans.