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Pharmacology based toxicity assessment : towards quantitative risk prediction in humans

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CHAPTER 2

Scope and intent of investigation

Historically, the evaluation of safety pharmacology and toxicity of drugs has largely relied on research in animal models, of which results have been used to extrapolate to potentially harmful events in humans. The research in these models has been developed to evaluate specific toxicological endpoints, (such as oral, dermal and ocular toxicity, immunotoxicity, genotoxicity, reproductive and developmental toxicity and carcinogenicity) rather than specifically designed to understand the exposure response relationships associated with the anticipated adverse event or toxicity (1). Furthermore, even if one considers the information obtained from these experiments useful, they are low throughput and inconsistently predictive of human pharmacology and pathophysiology. Some of these limitations persist in spite of the recognition of toxicokinetics as an important part of the safety assessment (2-3).

More recently, several major new initiatives have begun to utilise *in vitro* methods and a variety of new technologies to develop *in vitro* signatures and computational models predictive of *in vivo* response. These initiatives provide insight and tools to identify a battery of *in vitro* assays to detect perturbations in cellular pathways that are expected to contribute to or result in adverse health effects (4,5). Furthermore, these initiatives represent a welcome movement away from traditional *in vivo* high-dose hazard studies (6,7).

Despite such a continuing improvement in methods to characterise the safety and toxicity of novel medicines, uptake of these new approaches by regulatory agencies remains limited, with quantitative pharmacology concepts still being rarely applied to address clinical and regulatory questions on the safety pharmacology and toxicity of a novel compound. Evidence of the relevance of such concepts has been highlighted with the introduction of structure-activity relationships (SAR) in the absence of adequate toxicity data on the

chemical under certain circumstances, such as when the extent of exposure of humans is extremely low and toxicokinetic data cannot be easily generated (8). Clearly, the lack of a stronger pharmacological basis for the assessment of safety has prevented the implementation of a model-based approach aimed at the characterisation in a strict quantitative manner of the relationship between drug exposure and effects. To date, efforts have been limited to physiologically-based pharmacokinetic modelling, but it is mostly applied to environmental toxicology, rather than to pharmaceutical R&D (9).

Irrespective of the urgently needed changes in regulatory guidance, methodologies that support the translation and prediction of safety pharmacology and toxicity in humans are still required. To this purpose, more than novel experimental protocols and technologies are required. We strongly believe in integrative approaches that enable efficient use of available evidence and facilitate the assessment of pharmacokinetic-pharmacodynamic relationships. Of special importance is the possibility to evaluate and predict long term or rare adverse events, which continue to contribute to high attrition in drug discovery and development (10). In light of the known limitations of current experimental protocols and the implications they represent for hazard characterisation in humans, a range of different approaches is necessary to ensure that the appropriate endpoint is detected and risk evaluated in a precise and accurate manner.

The scientific and regulatory communities should acknowledge that most toxicity tests, as currently designed, are aimed solely at hazard identification at supratherapeutic levels. Data produced using current testing guidelines are not always suitable for robust mathematical exposure–response modelling. We recognise therefore that adequate characterisation of the exposure–response relationship requires a number of doses giving a range of different response levels. On the other hand, mathematical modelling of the exposure–response relationship would represent an important improvement to the risk assessment process.

Here we tackle a number of issues that need to be considered during the course of drug discovery and development to ensure more efficient use of the evidence on safety

pharmacology and toxicity which is generated. Four central questions will form the basis for the work to be presented in the subsequent chapters in this thesis:

1. Can current experimental protocols for safety pharmacology and toxicology evaluation be optimised to support the characterisation of pharmacokinetic-pharmacodynamic relationships?

2. Does a meta-analytical approach based on nonlinear mixed effects modelling provide more precise and accurate estimates of safety thresholds than current methodologies?

3. Can mechanism-based models be used for accurate inferences about safe drug exposure for low frequency, delayed (long term) or rare adverse events?

4. Should biomarkers be used in conjunction with pharmacokinetic data to enable accurate estimation of the safe drug exposure (and consequently of safety thresholds) during chronic therapy?

Our work is presented in a way that both conceptual and practical issues are addressed concurrently. After revisiting the requirements for the implementation of quantitative pharmacology concepts in the evaluation of safety pharmacology and toxicology, we highlight how existing protocols should be redesigned to obtain accurate results from the modelling and emphasise that an appropriate design might even result in a reduction in the total number of animals studied. Moreover, we show that biomarker data may allow translation of the external dose to an internal dose (or target-organ dose), as it reflects a compounds pharmacology. In fact, using naproxen as a paradigm compound for the acute and chronic effects of cyclo-oxygenase inhibition, we explore how biomarkers could be used to provide a full pharmacologically-based exposure-response model, i.e., a PBPKPD model. Our endeavour is complemented by further insight into the implications of modelling for risk prediction purposes, as described by logistic, hazard models. Clear recommendations are provided about the requirements for future refinements regarding the characterisation of

exposure–response relationships, which need to account for the extent of uncertainty and variability in modelling and simulation output.

Section I: General introduction

In **Chapter 1**, we have described the problems with existing practices from a methodological point of view and highlighted the value of mechanism-based PKPD modelling as a tool for the evaluation of safety pharmacology and toxicology. From a methodological perspective, we show that the parameterisation of drug exposure and available metrics of risk are often justified by historical precedent rather than by an informed scientific rationale. These measures are assumed to be predictive of drug effects in humans, despite the fact that in many cases known pharmacokinetic and pharmacological drug properties contradict such assumptions. Evidence clearly shows that empirical protocols remain primarily descriptive rather than explanatory of the observed phenomena and are therefore unsuitable for extrapolation, which is an important point to consider when analysing and interpreting safety pharmacology and toxicology data. Moreover, statistically, the use of point estimates and thresholds prevents understanding of the consequences of between subject variability and identification of at-risk subpopulations. Additionally, type I and II errors are also not accounted for in the design or analysis of toxicity data, both of which are critical informed decision making.

A shift in paradigm is proposed to 1) ensure that pharmacological concepts are incorporated into the evaluation of safety and toxicity; 2) facilitate the integration of historical evidence and thereby the translation of findings across species; and 3) promote the use of experimental protocols tailored to address specific safety and toxicity questions. Three important components have been identified, which will form the framework proposed throughout this thesis., namely, model based optimisation of experimental design, data integration, and incorporation of biomarkers as a way towards the implementation of a

pharmacology-based approach for the characterisation of safety and toxicity in drug discovery and development.

Of particular interest for us is to demonstrate that inefficiencies in the experimental design violate the principle of the 3 Rs (reduction, refinement and replacement) (11,12). Optimality concepts are available that could be implemented even when terminal sampling procedures are used, as is the case of histopathological measures. Using examples, we show that the poor predictive value of experimental data reflects the failure in anticipating the biological consequences of target engagement, i.e., in establishing the correlation between target-related events and drug exposure, as defined by the evidence of pharmacokinetic-pharmacodynamic (PKPD) relationships.

Based on the requirements for the implementation of a pharmacology-based approach, specific issues have been identified which underpin the scope and intent of the investigations described here **in Chapter 2**. Nonlinear-mixed effects modelling will be recommended as a tool for protocol optimisation and knowledge integration (i.e., evidence synthesis). Its use in the evaluation of pharmacokinetics and pharmacokinetic-pharmacodynamic relationships has enabled the advance of quantitative approaches in pharmacological research in recent decades. As shown in the subsequent chapters of this thesis, comparable benefits can be anticipated for the assessment of safety and toxicity.

The overall focus of the work presented in the following sections of this thesis is therefore to illustrate how the proposed methodology can be applied prospectively during the evaluation of a novel molecule in the early stages of development. We also attempt to demonstrate the need and added value of an integrative approach to predict potential long term AEs with respect to performance metrics commonly used in safety pharmacology and toxicology experiments. Where possible, proposals to amend study protocols are kept to a minimum to facilitate acceptance of the proposal by industry and regulatory bodies.

Section II: Conceptual framework

In the section, different aspects, including advantages and limitations of a model-based approach are evaluated. Of interest is the fact that despite the increased attention to the importance of toxicokinetics, the extrapolation and prediction of a safe exposure range in humans from preclinical experiments continues to be based on the assessment of empirical safety thresholds, in particular the no observed adverse effect level (NOAEL), which is a *qualitative* indicator of acceptable risk. In addition, pharmacokinetic data generated from different experiments are not evaluated in an integrated manner, whereby drug disposition (e.g., clearance) can be described mechanistically or at least compartmentally in terms of both first and zero order processes. As a consequence, safety thresholds are primarily derived from inferences about the putative pharmacokinetic profiles in the actual treatment group. Such an experimental setting has far reaching consequences for the assessment of risk, given the assumption that inter-individual differences are implied to result from residual variability. Pharmacokinetic and pharmacokinetic-pharmacodynamic parameters are treated as point estimates. Factors such as within- and between-subject variability or uncertainty in estimation are not accounted for. This is further complicated by another major limitation in the way exposure is described by naïve pooling approaches, i.e., the impossibility to accurately derive parameters such as cumulative exposure, indirect or delayed effects, which may be physiologically more relevant depending on type of drug and the mechanism of action.

Therefore, focus is initially given to the opportunities for optimisation of experimental protocols supporting the characterisation of pharmacokinetic properties at therapeutic and supratherapeutic levels. In **Chapter 3**, we show that the estimation of safety thresholds such as the NOAEL can be optimised (13). Using simulation scenarios in which hypothetical compounds with different disposition properties are evaluated, our analysis shows the feasibility and relative performance of a model-based analysis for the characterisation of systemic exposure as compared to empirical, non-compartmental analysis (NCA) methods

currently used in general toxicity protocols. Simulation scenarios are used to illustrate which changes are required in experimental protocols with respect to standard non-compartmental analysis. Expected bias and precision of parameters of interest, such as systemic exposure (AUC) and peak concentrations (C_{MAX}), are then computed with both methodologies. In addition, we also assess the predictive accuracy of cumulative exposure estimates up to three months beyond the study duration. It should be noted that such an extrapolation represents an important advantage of model-based methods, which cannot be derived by descriptive methods such as non-compartmental analysis. Overall, the scope of this evaluation was to show that, despite the need for an iterative process, modelling provides the basis for experimental protocol optimisation.

Given the assumption of unbiased parameter estimation when using a model-based approach for the characterisation of pharmacokinetic properties, a natural question arises with respect to the principle of the 3 Rs in pre-clinical research. Irrespective of the availability of alternative methods that allow evaluation of drug disposition properties *in vitro*, can experimental protocols be optimised to ensure a significant reduction in the number of animals required, whilst still providing sufficient estimation precision for measures of exposure such as AUC and C_{MAX}?

This question is addressed in **Chapter 4**, where an important methodological challenge is overcome, namely the possibility to optimise secondary pharmacokinetic parameters such as AUC and C_{MAX}. In contrast to existing optimality software and algorithms, which support optimisation of experimental design with respect to primary parameter precision, we show that secondary parameters can be optimised without the resource-intensive procedures imposed by D-optimality. Using a range of hypothetical drugs with different pharmacokinetic profiles, we illustrate the implementation of optimisation procedures to select sampling times and define the minimal number of animals per treatment group. By combining the expected Fisher information matrix (FIM) with simulations from uncertainty, this exercise ultimately shows that the precision of secondary parameters can be assessed and minimally sufficient designs obtained, in line with the principle of the 3 Rs. The method

is computationally inexpensive and can provide potential savings to numbers of animals without compromising study objectives.

Still within the scope of protocol optimisation, we also explore the implications of introducing biomarkers into the evaluation of a drug's safety toxicity profile, as biomarkers of pharmacological activity can be crucial for the prediction of long term adverse events and toxicity. In contrast to traditional protocols, which imply a direct relationship between observed systemic exposure and adverse events, in **Chapter 5** we apply a model-based approach to characterise the PKPD correlations and the time course of biomarker responses associated with long-term safety. Our evaluation also compares the analysis of biomarker data based on standard non-compartmental methods. In brief, we propose the collection of biomarkers at the scheduled pharmacokinetic sampling points to facilitate the characterisation of pharmacokinetic-pharmacodynamic relationships.

Study data are simulated for four hypothetical drugs, each with a different mechanism of delayed toxicity. For the purposes of our evaluation, delayed toxicity was parameterised in terms of i) an indirect response mechanism, ii) an indirect response mechanism preceded by biophase equilibration, iii) cumulative effects as a consequence of chronic dosing and iv) formation of a toxic metabolite after repeated dosing. Given the often unknown mechanism of toxicity, model misspecification is also considered to ensure that accurate conclusions are drawn from experimental protocols. Finally, bias and precision of parameter estimates were used as metrics of interest to compare model-based and non-compartmental methods.

The utility of model-based approaches to predict the risk of adverse events from preclinical toxicology protocols is subsequently explored in **Chapter 6**, where pharmacokinetic, biomarker and adverse event data are integrated into a PKPD model. In this investigation, simulation scenarios are used to generate drug-induced adverse events for reversible and irreversible drug effects according to three different pharmacological mechanisms (direct, indirect, and irreversible binding). To ensure real-life conditions, assumptions are made with regard to 1) the presence of background adverse events, including the situation in which

drug-induced and background adverse events are indistinguishable from each other, 2) events occur with low frequency, including rare events, 3) the symptoms evolve over time but can only be detected once per animal during histological examination and 4) adverse events are described by binary data.

Whereas typical toxicology experiments are designed to show evidence of safety thresholds, it can be anticipated that they may not fully support the identification of the underlying mechanisms for adverse drug reactions. Therefore, we show the importance of prior information and more specifically of background rates from placebo and control-treated animals. We also make the effort to quantify model and parameter uncertainty as the basis for subsequent risk assessment. At the same time, we show the technical challenges for characterising exposure-response relationships, which make the validity and reproducibility of models derived by empirical experimentation questionable for predictive purposes.

Section III: Case study and practical application

The third part of the thesis aims to illustrate the implementation of experimental protocols suitable for model-based analysis. Given the ongoing debate of the benefit-risk balance of chronic treatment with non-steroidal anti-inflammatory drugs, naproxen is used as a paradigm compound with known acute and chronic toxicities. Naproxen is a non-selective cyclo-oxygenase inhibitor, whose activity results in the suppression of pro-inflammatory mediators such as prostaglandins and thromboxanes (14). By considering the requirements for a suitable experimental protocol, we also attempt to identify practical challenges and difficulties that one may face for the prospective use of the methodology.

From a clinical pharmacology perspective, the rationale for selecting naproxen is based on the differences in housekeeping function of both isozymes and their contribution to the inflammatory response in acute and chronic inflammatory conditions (15-17). Unfortunately, at present the dose selection of COX inhibitors disregards whether maximum, long-lasting blockade of either enzyme systems is strictly required for anti-inflammatory, analgesic

response and how its pharmacology relates the observed adverse events (18). These considerations become essential when evaluating the side effects associated with long term use of COX inhibitors, which include gastric and cardiac adverse events. Whilst the lack of selectivity of naproxen and the evidence for distinct mechanisms underpinning acute effects (such as bleeding and ulceration) and long term effects (such as renal and cardiovascular damage) have evolved over the years and might not have been understood at time of the development of the compound, such understanding makes it quite didactic in that it demonstrates how human safety and toxicity may require characterisation of drug effects at exposure levels corresponding to the therapeutic doses. Toxicity, and in particular, long term safety is not a matter of supra-therapeutic exposure: it may be determined by time-dependent pharmacological activity.

Using a typical toxicology protocol in rats, in **Chapter 7** naproxen, a non-selective cyclo-oxygenase inhibitor is used as paradigm compound to demonstrate the concept of biomarker-guided safety assessment (19-21). Using pharmacokinetic-pharmacodynamic techniques, we subsequently illustrate how modelling and simulation techniques can be used to ensure accurate estimation of the safe dose levels of naproxen after chronic exposure. Furthermore, the pharmacokinetics of naproxen is evaluated in conjunction thromboxane (TXB_2) and prostaglandin (PGE_2) over short, moderate and long-term treatment. It is assumed that gastrointestinal bleeding is due continuous COX-1 inhibition, whereas ulceration results primarily from the suppression of COX-2, which is known to have an important role in the repair of gastric mucosa. PK and biomarker findings are then integrated with experimental data from historical protocols and published literature to ensure characterisation of drug properties at putative therapeutic levels. From a methodological perspective, it is our endeavour to quantify the impact of nonlinearity in pharmacokinetics and in biomarker response. Given the wealth of clinical data from the published literature, we also take the opportunity to evaluate the predictive value of nonclinical findings and explore whether species differences exist for naproxen effects on TXB_2 and PGE_2.

This evaluation is complemented in **Chapter 8** by further integrating the histological data obtained at completion of treatment to the observed biomarker effects. Here we emphasise the need for evidence synthesis to quantify and explain the risks associated with long term drug exposure. Clearly, efforts are required to ensure the availability of tissue- and mechanism-specific data for accurate interpretation of acute and long term safety findings. Such an objective may be hampered by the use of empirical experimental protocols, as they render the extrapolation of findings across species rather difficult, preventing accurate translation of the pharmacological properties to man. In the current investigation we show therefore how pharmacokinetic-pharmacodynamic (PKPD) modelling can be used to unravel the relationship between chronic drug exposure, pharmacodynamic effects and overt symptoms and signs. The concept is illustrated by the correlation between naproxen concentrations, PGE_2 and TXB_2 inhibition, and gastric ulceration in rats. Through the use of bootstrapping procedures in combination with covariate analysis, we show how model diagnostics can be used for model selection as well as for potential identification of the explanatory factors for the observed gastric ulceration.

Section IV: Conclusions and Perspectives

An overview of the results and conclusions drawn from the various chapters is provided in **Chapter 9.** Most importantly, recommendations are provided for physiologically based quantitative toxicity assessment. Here we also summarise the answers to the initial questions set up at the beginning of this chapter, which underpin the research developed throughout the thesis. We anticipate that the examples used in Section II will make clear that there are numerous opportunities for optimisation of experimental protocols for safety pharmacology and toxicology. The approach should also shed light on the advantages of including biomarkers and characterising PKPD relationships, instead of relying solely on safety thresholds.

Lastly, the issues identified in the various simulation scenarios and the challenges experienced during the implementation of an integrated experimental protocol are discussed. Our findings should make clear that inferences about safe exposure as well as the risk associated with long term use of a compound cannot be achieved by scattered empirical experimentation. Given the increased relevance of evidence synthesis as the basis for decision-making within regulatory and clinical settings, we expect that some of the meta-analytical elements presented across the various simulation scenarios will become embedded into daily practice in safety pharmacology and toxicology. Irrespective of the advancements in the understanding of the mechanisms of toxicity, we envisage that a pharmacology or biomarker-based approach will always be required to allow accurate inferences about safe drug exposure for low frequency, delayed (long term) and rare adverse events. Future perspectives are then presented taking into account ongoing developments in the field of systems pharmacology and its relevance for the prediction of drug toxicity and risk assessment in humans. The work is concluded with a new question being asked with regard to the scientific and ethical basis for current experimental designs in toxicology.

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