Mind the time : 24-hour rhythms in drug exposure and effect

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CHAPTER

General discussion

8

A thorough understanding of the physiological processes that determine a drug's exposure and effect is required to address the challenges encountered during the development or optimisation of new and existing drug therapies. Although rarely considered by the pharmaceutical industry or clinicians, 24-hour rhythms in physiology can potentially influence the pharmacokinetics and pharmacodynamics of drugs. In **Chapter 1**, the current state of the field of chronopharmacology, which aims to characterize the influence of daily physiological rhythms on drug treatments, was reviewed. Although this field has existed for decades, we identified several methodological issues in the current body of literature that often precludes implementation of chronopharmacological principles in clinical practice. In general, it was found that a systematic approach to analyse and integrate the data obtained within this field is currently lacking. Therefore, the aim of this thesis was to develop a more structured approach to study the effect of 24-hour variation on the exposure and effect of drugs.

As explained in **Chapter 2**, this approach involves (1) the use of probe drugs, (2) prospectively designed studies optimally suited to investigate chronopharmacology, and (3) the application of pharmacokinetic-pharmacodynamic modelling for data analysis. Here, this approach is discussed in the context of the results presented in **Chapter 3-7**. Subsequently, the clinical implications and future perspectives of this research are considered.

THE USE OF PROBE DRUGS IN CHRONOPHARMACOLOGY

In theory, the exposure and effect of each drug could be influenced by daily rhythms in physiological processes in a unique manner. However, considering the vast amount of drugs currently used in clinical practice, it is neither feasible nor desirable to study the effect of dosing time on all of these drugs separately. Therefore, a key characteristic of the approach described in this thesis was the use of probe drugs to study the effect of 24-hour variation in specific physiological processes on their pharmacokinetics or pharmacodynamics. Midazolam was used as a CYP3A-substrate in the clinical trial described in **Chapter 3**. The rationale for using levofloxacin as a model compound in **Chapter 4** and **Chapter 5** was two-fold. Characterized by complete absorption and very limited metabolism, levofloxacin is an ideal compound to investigate solubility- and permeability-independent absorption and passive renal elimination. Additionally, as an inhibitor of cardiac hERG channels, it was used to study drug-induced QT prolongation. In **Chapter 6**, quinidine was chosen as a model compound to investigate P-glycoprotein mediated transport in the brain. Finally, in **Chapter 7**, we used morphine as a substrate of P-glycoprotein as well as of probenecid-sensitive transporters such as multidrug resistance-associated proteins (mrp). In addition to enhancing our understanding of morphine brain distribution, we gained insight into UGT2B7-mediated metabolism by the measurement of the plasma concentrations of morphine's metabolite M3G. This way, we acquired a substantial body of knowledge of the 24-hour variation in these physiological processes, which will be discussed here.

Drug absorption

Most drugs are administered orally and need to be absorbed before reaching the systemic circulation. In its most simple form, drug absorption involves the passive diffusion of drug molecules across the lipid bilayers of the intestinal epithelium, the extent of which is influenced by a drug's permeability and solubility. We studied 24-hour variation in the rate and extent of drug absorption in **Chapter 3** and **4**.

In **Chapter 3**, a randomized crossover study is described that involved the administration of an oral (2 mg) and intravenous (1 mg) dose of midazolam to 12 healthy volunteers at six different time-points using a semi-simultaneous dosing regimen. Previous clinical studies into the effect of dosing time on the pharmacokinetic parameters of midazolam did not yield consistent results. While two studies found significant diurnal variation in midazolam pharmacokinetics after intravenous infusion (Klotz and Ziegler, 1982; Tomalik-Scharte et al., 2014), another study did not observe these differences (Klotz and Reimann, 1984). Furthermore, two studies addressed the effect of time of administration on midazolam pharmacokinetics after an oral dose (Klotz and Ziegler, 1982; Koopmans et al., 1991), which both observed that the absorption of the drug is not affected by the dosing times employed in the studies. Taken together, these studies exemplify the issues that were raised in Chapter 1, including the use of a limited number dosing times and suboptimal statistical methods. By performing the trial described in **Chapter 3**, we could identify the absorption and clearance parameters separately and construct a detailed profile of the variation over the 24-hour period. Our findings indicate that oral bioavailability showed significant 24-hour variation that could be described by a sinusoidal function with a peak at 12:14 in the afternoon and a relative amplitude of 14.2%. In combination with a 1.41 increase in the absorption rate constant at 14:00, this results in a higher exposure to this drug after oral dosing in the morning and afternoon, compared to oral dosing in the evening and night.

Because we found in **Chapter 3** that dosing time has the largest effect on processes related to drug absorption, this was further investigated in **Chapter 4**. As discussed in Chapter 1, most processes that influence the rate of absorption are more active during the morning compared to the evening, suggesting enhanced absorption during this time period. Indeed, there are indications that the absorption of some lipophilic drugs is faster in the morning (Baraldo, 2008). In **Chapter 4**, the daily variation in the rate of absorption of levofloxacin, a drug characterized by high solubility, high permeability, and minimal metabolism, could be very accurately determined. Substantial variation was identified in the absorption rate of levofloxacin with an amplitude of 47% and a peak at 8:00 in the morning.

Levofloxacin is used in the clinic as an antibiotic to treat a variety of bacterial infections. As a concentration-dependent antibiotic, levofloxacin's exposure, measured by the area under the curve (AUC), or the maximal concentration (C_{max}) determine its clinical effectiveness (Drusano, 2004; Preston et al., 1998). The simulations performed in **Chapter 4** indicate that the AUC and C_{max} are not significantly affected by dosing time despite the substantial variation in the absorption rate constant. Therefore, we concluded that, in clinical practice,

levofloxacin can be dosed without taking into account the time of day, at least in terms of its pharmacokinetic parameters.

Drug distribution to the brain

Although drug concentration-time profiles in plasma are typically used to assess the relationships between drug exposure and effect, it is the drug concentration at the target site that ultimately drives its effect (De Lange, 2013). Especially for drugs targeted at the brain, plasma concentrations are a poor predictor of target site concentrations because the blood brain barrier (BBB) restricts the entry of drugs to the brain. Understanding the mechanisms underlying the effect of drugs targeted to the brain therefore necessitates local measurement of drug concentrations. In **Chapter 6** and **7**, we investigated 24-hour variation in processes involved in the distribution of drugs in the brain using the rat as a pre-clinical animal model.

In the first part of **Chapter 6**, quinidine was administered intravenously at six different time-points across the 24-hour period to assess P-gp mediated distribution in the brain (Kusuhara et al., 1997; Sziráki et al., 2011). By measuring the quinidine concentration in plasma and brain tissue, it was found that the exposure to this drug in the brain is affected by time of drug administration. When the animals were pre-treated with the potent and selective P-gp inhibitor tariquidar (Mistry et al., 2001), this dosing time-dependent effect was abolished, suggesting that 24-hour variation in P-gp activity is responsible for the observed effect.

In the second part of **Chapter 6**, the microdialysis technique was used to obtain unbound quinidine concentrations in ECF and cerebral spinal fluid (CSF) after drug administration at the two time points that showed the largest difference in brain concentrations in the first part of the study. Microdialysis is currently the only available technique to measure unbound extracellular fluid (ECF) concentrations in the brain over time (De Lange, 2013). Subsequently, the systems-based pharmacokinetic model developed by Westerhout et al. (2013) was applied to the data to investigate the effect of dosing time on the pharmacokinetic parameters of quinidine in the presence and absence of functional P-gp activity. This analysis showed that the variation in brain concentrations could be described by higher activity of P-gp-mediated transport from the deep brain compartment to the plasma compartment during the active period. Furthermore, CSF flux was higher in the resting period compared to the active period. This study showed that dosing time is a considerable source of variation in the distribution of quinidine, and possibly other P-gp substrates, in the brain and suggests that taking into account time of day is a way to optimize drug treatments targeted at the brain.

Within the study design used in **Chapter 6**, we could not account for the effect of sleep on our results. Sleep, independent of circadian rhythmicity, was recently shown to have a profound influence on the clearance of potentially toxic molecules from the brain through an increase in interstitial fluid fluxes mediated by the glymphatic system (Jessen et al., 2015; Xie et al., 2013). Although the implications of this finding on the clearance of drugs from

the brain have yet to be determined, it may be an explanation for the increased CSF flow during sleep that we observed in **Chapter 6**. However, with regard to the 24-hour variation observed in P-gp activity, it was recently found using PET imaging that P-gp function in rats exhibits a 24-hour rhythm independent of sleep (Savolainen et al., 2015), suggesting that the observed 24-hour variation in P-gp mediated transport to brain tissue in **Chapter 6** is not affected by the sleep/wake state of the animal.

In **Chapter 7**, the effect of dosing time on the systemic pharmacokinetics and brain distribution of morphine was investigated. The possibility that daily variation in the analgesic effect of morphine is caused by fluctuations in the pharmacokinetics of morphine was already raised by Lutsch and Morris (1971), who suggested that the daily analgesia pattern produced by morphine could be due to rhythmic variation in the detoxification and/or distribution of morphine, or could result from variable permeability of the blood-brain barrier during the 24-hour period. This would result in 24-hour variations in the quantity of morphine arriving at and reacting with its receptor (Lutsch and Morris, 1971). Although the absorption of morphine after oral administration is influenced by dosing time (Dohoo, 1997; Gourlay et al., 1995), the study described in **Chapter 7** was the first to directly test the hypothesis that the brain distribution of morphine displays 24-hour variation.

To quantify the effect of time of day on morphine brain distribution, a pharmacokinetic model was developed based on the experimental data. It was found that the efflux of morphine from the brain ECF compartment into the circulation can be best described by a sinusoidal function with 24-hour and 12-hour harmonic terms. The shape of this cosine function is characterized by troughs around the light/dark phase transitions. In combination with the rhythms found in the active clearance of morphine and its metabolite M3G from the system, this results in highest morphine concentrations in brain in the early dark phase. This finding is in line with previous research showing that the highest analgesic effect of morphine in the dark phase (Bornschein et al., 1977; Cui et al., 2005; Lutsch and Morris, 1971; Morris and Lutsch, 1967; Yoshida et al., 2003), although it should be noted that others reported the highest effect in the light phase (Güney et al., 1998; Rasmussen and Farr, 2003) or no effect of dosing time (Kavaliers and Hirst, 1983; Oliverio et al., 1982). Future research should focus on establishing the link between the rhythm in brain exposure to morphine and its analgesic effect through PK-PD modelling.

Drug elimination

Important pathways in the elimination of drugs are renal excretion or metabolic conversion. In the clinical trial described in **Chapter 4**, glomerular filtration rate (GFR), measured by inulin clearance, was determined at six different time-points across the 24-hour period in twelve healthy subjects. Time of day significantly affected GFR, with the highest value at 9:00 in the morning and the lowest value at 01:00 at night, which is in line with previous research (Buijsen et al., 1994; Koopman et al., 1989; Voogel et al., 2001). However, the relative difference in GFR between these two time points was 9%, which is too small to be clinically relevant. Likewise, in **Chapter 4**, we could not identify significant 24-hour rhythmicity in

the clearance of levofloxacin, a drug mainly eliminated through passive renal elimination (Fish and Chow, 1997). Hence, 24-hour variation in kidney function minimally affects the pharmacokinetic profile of levofloxacin at different time-points throughout the day and night.

In contrast to levofloxacin, the contribution of renal elimination of midazolam is negligible, with the percentage of midazolam that is excreted unchanged in urine being around 0.02% (Smith et al., 1981). Instead, the primary pathway of midazolam elimination is hepatic metabolism by CYP3A enzymes (Thummel et al., 1996). In **Chapter 3**, a small but significant 24-hour rhythm was identified in the clearance of midazolam with a peak at 18:50 in the evening. As midazolam has an extraction rate of 35% (Tsunoda et al., 1999), this rhythm is probably not due to variation in hepatic blood flow (Lemmer and Nold, 1991), but rather to 24-hour variation in hepatic CYP3A activity (Ohno et al., 2000; Takiguchi et al., 2007). Of note, CYP3A4 transcript levels in serum-shocked cultured human hepatic cells vary over the 24-hour period by more than threefold, while we and others find a rhythm in midazolam clearance with a relative amplitude of 10-15% (Tomalik-Scharte et al., 2014). Additionally, simulations revealed that this rhythm has a limited effect on the concentration-time profiles of midazolam in plasma after intravenous dosing. This demonstrates the need for translational approaches that directly try to establish a mechanistic link between changes in gene expression with functional characterization of physiological processes.

Daily variation in drug-effect relationships

In addition to daily rhythms in pharmacokinetics, the relationship between drug concentration and the desired therapeutic effect and/or adverse side-effects may also show 24-hour variation. As discussed in **Chapter 1**, there is ample evidence that the effect or toxicity of many drugs varies over the 24-hour period. However, the quantification of these relationships through PK-PD modelling is not commonly performed. In **Chapter 5**, we investigated whether the extent of drug-induced QT prolongation depends on the time of day and show that PK-PD modelling is a powerful tool to assess 24-hour variation in drug-effect relationships.

Drug-induced QT prolongation, a sign of delayed ventricular repolarization manifested on the electrocardiogram (ECG), is a common side-effect of many different types of drugs that can result in potentially fatal ventricular arrhythmias (Kannankeril et al., 2010). The ICH E14 guidelines, formulated by the International Conference on Harmonisation (ICH) and subsequently adopted by the regulatory agencies in the US, Europe and Japan, stipulate the execution of a "thorough QT/QTc" (TQT) study for all new drugs prior to approval (ICH, 2005). This involves a dedicated clinical trial in which the effect of a placebo, a therapeutic and a supra-therapeutic dose on the QT interval is carefully evaluated. The potential of a drug to prolong the QT interval is determined by calculating the baseline-subtracted mean change in the QT interval at multiple time-points after dosing compared to placebo. If the upper bound of the 95% confidence interval exceeds 10ms, the TQT study is regarded positive, i.e. it confirms the null-hypothesis that the drug has an effect on the QT interval. The outcome

of a TQT study has large impact, as a positive trial requires substantial ECG monitoring during the subsequent phases of drug development (Darpo et al., 2015).

Levofloxacin prolongs the QT interval to a small extent (Taubel et al., 2010) through inhibition of hERG potassium channels (Kang et al., 2001). Since hERG channel blockade is the most common mechanism by which drugs prolong the QT interval, levofloxacin was used in Chapter 5 as a model compound to investigate whether drug-induced QT prolongation is influenced by time of day. Building upon the pharmacokinetic model developed in Chapter 4, we show that the sensitivity to levofloxacin varies considerably over the day. This variation was described by a two-harmonic sinusoidal function with a peak in the late afternoon and a trough in the early morning. In the context of studies that provided circumstantial evidence that drugs may alter the daily variation in the duration of the QT interval (Antimisiaris et al., 1994; Watanabe et al., 2012), **Chapter 5** is the first study to characterize and quantify this relationship.

The finding that the sensitivity to a QT-prolonging drug varies over the 24-hour period is relevant for the recent reports that provide a mechanistic link between circadian rhythmicity and the development of cardiac arrhythmias. In mice, circadian variation in QT interval duration is controlled by the transcription factor Klf15, which is rhythmically expressed in cardiomyocytes (Jeyaraj et al., 2012). This control is possibly exerted through the rhythmic induction of KChIP2 expression, a critical subunit involved in cardiac repolarisation. Aberrant expression of Klf15 results in a loss of rhythmic QT variation and enhances the susceptibility to ventricular arrhythmias (Jeyaraj et al., 2012). Furthermore, the clock gene Bmal1 regulates the expression of Scn5a, a cardiac voltage-gated sodium channel, and Kcnh2, the murine form of hERG potassium channel. In the absence of Bmal1, ventricular repolarization is altered (Schroder et al., 2013, 2015). These findings indicate that rhythmic transcription of cardiac ion channels may affect the electrophysiology of the heart. Future studies are warranted to investigate if and to what extent the rhythmic expression of ion channels provides an explanation for the time-of-day dependent changes in cardiac sensitivity to QT prolonging drugs.

In current practice, each occasion in a TQT study is conducted at the same time of day. In this way, the 24-hour variation in the baseline QT interval and the autonomic changes that occur during sleep can be controlled for (Browne et al., 1983; Extramiana et al., 1999). However, this approach relies on the implicit assumption that drug-induced QT prolongation is independent of time of day, potentially introducing a bias in TQT studies that might lead to an inaccurate assessment of the risk to patients who may take the drug at any time point (Dallmann et al., 2014). Our clinical trial simulation-based results presented in **Chapter 5** challenge this assumption by showing that the extent of levofloxacin-induced QT prolongation is heavily influenced by dosing time, with the largest effect of 1.73 [95% P.I. 1.56-1.90] ms per mg/L predicted after dosing at 14:00 and the smallest effect of -0.04 [-0.19-0.12] ms per mg/L after dosing at 06:00. Accordingly, we found that the predicted probability of a positive outcome of a dedicated QT trial varies considerably over the course of the day, with the highest probability observed after drug administration at 14:00 and

the lowest probability after drug administration at night (22:00, 02:00 and 06:00). Although future research is warranted to investigate whether these findings can be replicated with other QT-prolonging drugs under placebo-controlled conditions, it suggests that the time at which clinical trials are conducted may introduce bias in testing drug-induced QT prolongation. Hence, current drug safety evaluations may systematically misjudge the risk to patients by ignoring time of day.

DESIGN OF CHRONOPHARMACOLOGICAL STUDIES

An overarching feature of the studies described in **Chapter 3-7** is that they were prospectively designed to evaluate the effect of time of day on the pharmacokinetic and/or pharmacodynamic properties of various probe drugs. Among the many chronopharmacological studies cited in **Chapter 1** that used a limited number of dosing times and therefore may have missed the peak and trough of the parameters of interest, a key asset of the studies described in this thesis is the use of six dosing times equally distributed over the 24-hour period. As described in more detail below, this allowed us to precisely characterize the variation in pharmacokinetic or pharmacodynamic parameters over the 24-hour period through the use of mathematical modelling and simulation.

The studies described in this thesis were conducted under tightly controlled laboratory conditions. For example, the studies described in **Chapter 3, 4** and **5** were conducted in healthy male volunteers with an intermediate chronotype and a stable diurnal activity rhythm. This was assessed through a chronotype questionnaire, sleep diaries and actigraphy recordings prior to the study. During the study days, sleep disturbance was kept to a minimum and subjects wore eye masks during the night to prevent any disruptive effect of light exposure or sleep deprivation on physiological rhythms (Mullington et al., 2009). Additionally, the timing of food and water intake was tightly controlled to limit their potential influence (Singh, 1999). This approach allowed determining 24-hour variations in the pharmacokinetics and pharmacodynamics of drugs with as little confounders as possible. These dedicated clinical trials provide a basis for further research under more realistic conditions. In this regard, population-wide studies such as the Rotterdam Study are valuable sources of data that can be used to assess drug exposure and effect in typical patient populations under real-life conditions (Chain et al., 2013).

PK-PD MODELLING IN CHRONOPHARMACOLOGY

Pharmacokinetic-pharmacodynamic modelling is being increasingly used within the academia and pharmaceutical industry to guide decisions in drug discovery and development. In this thesis, PK-PD modelling was applied to the field of chronopharmacology, with special attention given to the means to identify 24-hour variation in pharmacokinetic and pharmacodynamic parameters.

In **Chapter 1**, it was put forward that diagnostic plots are an essential tool to facilitate the identification of 24-hour variation in the model parameters, although their use is rarely

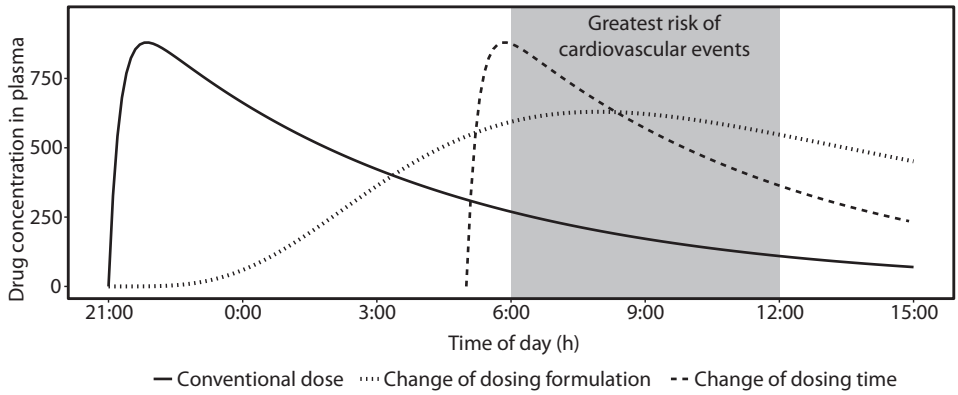

Figure 1 Modification of dosing regimen to provide optimal exposure at the desired time window. Administration of a conventional hypertensive drug before bedtime results in low drug concentrations in plasma during the time window during which the risk of cardiovascular events is greatest (solid line). Adjusting dosing time (dashed line) results in higher concentrations during this period but requires administration at an unpractical point of time. Adjusting the dosing formulation to a delayed onset sustained-release form (dotted line) provides favourable concentrations during the entire critical time-window and allows for a more practical dosing time. Image adapted from Wertheimer et al., 2005.

reported. In the PK-PD models that were developed in the context of the research described in this thesis, graphical evaluation of the improvement of fit has been instrumental. In **Chapter 3**, describing the bioavailability and the clearance of midazolam by sinusoidal functions with a period of 24 hours resolved the time-of-day dependent bias observed in the interoccasion variability, in addition to improving the fit of the model as judged by the decrease in objective function value (OFV). Likewise, accounting for the increase in the absorption rate constant at 14:00 reduced the bias in interoccasion variability in this parameter. Similarly, in **Chapter 4**, it was found that the distribution of interoccasion variability on the absorption rate constant of levofloxacin varied over the 24-hour period, which could also be described by a sinusoidal function. In **Chapter 5**, a large degree of interoccasion variability was present on QT0, the parameter that describes the QT interval at baseline, which was reduced to 0 after accounting for the time-of-day dependent changes in the levofloxacin-QT relationship. The distribution of residuals over time of day can also be used to visualize an improvement of fit (Lee et al., 2014). In **Chapter 5**, the bias in the distribution of the conditional weighted residuals with interaction (CWRESI) over time of day was removed after inclusion of the sinusoidal function on the levofloxacin-QT relationship. In **Chapter 7**, similar improvements were noted in the CWRESI distribution over time by describing the efflux from morphine from the brain to plasma by a sinusoidal function. These approaches to identify systematic 24-hour variation during the development of a pharmacokinetic and pharmacodynamic model can be applied to future chronopharmacological research.

Throughout this thesis, sinusoidal functions have been used to describe variation in pharmacokinetic and pharmacodynamic parameters, while, as discussed in **Chapter 1,** previous studies typically used dosing time as a covariate. It could be argued that using dosing time as a covariate is a less biased approach as it does not make assumptions regarding the shape of the variation. However, sinusoidal functions have several advantages

as it provides a continuous description of parameters over time. This allows for the simulation of dosing times and regimens that were not investigated in the original study. In **Chapter 4, 5** and **7**, the advantage of this approach was shown through the use of simulations. For example, in **Chapter 5**, we were able to show that dosing time may introduce bias in the predicted outcome of thorough QT-like studies. Additionally, the use of sinusoidal functions is helpful in the case of drugs with a long half-life, as the physiological processes underlying the pharmacokinetic parameters will continue to change after dosing.

Together, these strategies can be used to support decisions during the development of chronopharmacological models.

CLINICAL IMPLICATIONS

A key question that emerges from this thesis concerns the implications for drug development and clinical practice.

Chronopharmacology in drug development

Collectively, the studies described in this thesis have shown that time of day is a considerable source of variation in the pharmacokinetic and pharmacodynamic parameters of a drug that can be identified and precisely quantified through modelling and simulation. If not taken into consideration, time-of-day dependent variation can be easily mistaken for inter- or intraindividual variation. However, characterizing time of day during drug development is not only required to account for and understand this source of variation, it is also essential in order to benefit from systematic fluctuations in physiology. For example, the finding that P-gp mediated transport differs by more than two-fold between the day and night (**Chapter 6**), can be employed to optimize the exposure of P-gp substrates to the brain. Therapeutic P-gp substrates that have their target in the brain, such as those used for the treatment of neurological disorders, should be dosed at the time at which the exposure is maximal. P-gp

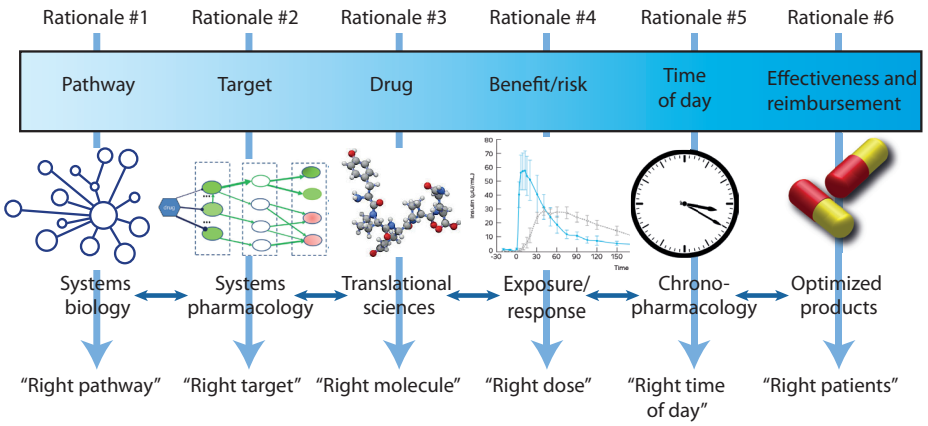

Figure 2 The integrated view of model-based drug discovery and development, expanded to include chronopharmacology. This paradigm, without rationale #5, was originally presented by van der Graaf and Benson (2011). The research presented in this thesis shows that this paradigm should be extended to include chronopharmacological considerations. Figure adapted from Milligan et al. (2013).

substrates that act systemically and that inflict adverse neurological side effects, should be dosed at the time at which the exposure to the brain is minimal. Modulating transporter function at the human BBB through pharmacological means has proven difficult (Kalvass et al., 2013). In this light, the changes that might occur under normal physiological conditions might be of interest. Therefore, future research is warranted to investigate to what extent the results presented in **Chapter 6** apply to human patients.

Additionally, the results presented in **Chapter 5** ask for more attention on dosing time-dependent effects during drug development as a means of reducing the risk to patients once a drug is released on the market. If the findings presented in this chapter apply to other hERG inhibiting drugs in a placebo-controlled clinical trial, this calls for a circadian testing policy to be adopted by drug developers, at least in the context of drug-induced QT prolongation (Dallmann et al., 2014).

Chronopharmacology in clinical practice

In a clinical setting, the aim of chronopharmacology is to time therapies such that the effect is maximal while the unwanted side-effects are minimal. This can be achieved in two ways: either by adjusting the administration time of conventional drug formulations and/or by altering the formulation (Figure 1) (Kaur et al., 2016). However, these strategies require accurate patient adherence, which may be an issue especially when drug are taken by patients at home (Smolensky and Peppas, 2007). Timing compliance has been reported to be particularly low. For example, the percentage of doses taken within an 8±1 h interval of a three times daily oral dose of an antibiotic was reported to be as low as 10.9% (Cals et al., 2008). In this light, worth noticing that morning compliance is generally higher than evening compliance (for example Kahook and Noecker, 2007; Kardas, 2004; Vrijens et al., 2008), although the opposite has been reported as well (Jonasson, 2000). Hence, the low degree of time-of-day dependent compliance should be taken into account when devising chronopharmacological dosing regimens.

One strategy to increase compliance would be proper training of the clinicians and pharmacists that instruct patients about optimal drug use (Kaur et al., 2016). However, another promising avenue for the field of chronotherapy is the development of programmable pumps and other advanced drug delivery systems that, for example, release drugs in response to circulating biomarker levels in the blood (Smolensky and Peppas, 2007).

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

In their seminal paper on systems pharmacology, van der Graaf and Benson (2011) stated that for modelling and simulation to achieve its full potential, the different stages of the R&D cycle should be integrated into an enhanced quantitative drug discovery and development paradigm that includes identification of the "right pathway, right target, right molecule, right dose and right patient". This thesis indicates that this paradigm should be extended to include the "right time of day" (Figure 2) and provides a framework for integrating of this

principle in the field of modelling and simulation.

As shown in Figure 2, full implementation of chronopharmacology during drug discovery and development requires integration of this field among the other disciplines employed in the field of pharmacology. Therefore, translational studies that aim to unravel the underlying mechanisms will be instrumental to the advancement of chronopharmacology in the clinic. Recently, it was reported that the majority of best-selling drugs directly target the product of a gene that is rhythmically expressed, suggesting that the effect of these drugs depend on the time of day (Zhang et al., 2014). Together with the systematic approach presented in this thesis, this opens up new avenues for further research into the effect of time on the exposure and effect of drugs that can bridge the gap from bench to bedside.

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