The 'free drug hypothesis' : fact or fiction?

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Chapter 1

The 'free drug hypothesis': Fact or fiction?; Scope and outline of this thesis

1.1 Background and objective

The objective of the investigations described in thesis was the development of a theoretical framework for prediction of the role of plasma protein binding as a determinant of in vivo drug effects. A systems pharmacology approach was chosen for the investigation of the influence of plasma protein binding on the pharmacodynamics, since plasma protein binding may simultaneously affect both the pharmacokinetics (PK) and the pharmacodynamics (PD) of drugs. The theoretical basis of the influence of (alterations in) plasma protein binding on PK is well-established and clearly defined as being either restrictive or non-restrictive for the clearance and volume of distribution. It has been shown that changes in plasma protein binding may lead to alteration in the drug exposure in terms of the total plasma concentration versus time profile. For the PD plasma protein binding may influence multiple parts of the cascade leading from drug administration to drug effect (e.g. PK, target site distribution and target binding) (Danhof *et al.,* 2007; 2008). As such investigating the influence of plasma protein binding on PD is more complex than for the PK and, therefore, the investigations require a mechanism-based PKPD modelling approach based on the simultaneous assessment of the influence of plasma protein binding on the PK and PD.

1.2 Scope and outline

Theoretical framework for prediction of the influence of plasma protein binding on PD
In **section I**, **chapter 2** an *in silico* approach was chosen to examine in a quantitative manner the influence of plasma protein binding on receptor occupancy. Meaningful parameter estimates for the drug-target and drug-protein binding affinities for the simulations were obtained from literature to provide insight in the "direct" competition between binding at plasma protein and the receptor. The results indicate that plasma protein binding will indeed be restrictive for the PD of most drugs under conditions of rapid equilibrium. Non-restrictive protein binding with regard to the PD is possible but is only observed for drugs with a very high affinity for the target compared to the affinity for the plasma protein (i.e. >1000-fold difference in binding affinity).

β-adrenoceptor antagonists
β-adrenoceptors antagonists (β-blockers) were selected as model compounds in the investigations described in the **sections II** and **III**, since they are considered uniquely suitable drugs for the investigation of the relation between specific drug characteristics on drug action in vivo. As a class, the β-blockers are quite diverse, because they display a high range of values in plasma protein binding and also differ substantially in their affinity for binding to β-adrenoceptors (Johnsson and Regardh, 1976; Riddell *et al*., 1987; Mehvar and Brocks, 2001; Singh, 2005). In addition, the pharmacology of β-blockers and their mechanism of action are well established. β-blockers are antagonists and as a consequence the pharmacological effect on heart rate is directly related to receptor occupancy. Heart rate can, hence, be used as a biomarker for receptor binding. A more practical advantage for the use of β-blockers is the readily available PD endpoint in both humans and laboratory animals (Wellstein *et al*., 1987; Piercy, 1988; Kendall, 1997). The β-blocker, S(-)-propranolol, has been frequently used in the research of plasma protein binding . Most studies indicate that the free concentration is the main determinant of drug effect for this β-blocker (Terao and Shen, 1983; Yasuhara *et al*., 1985; Belpaire *et al*., 1986; Chindavijak *et al*., 1988). The aim of the currently presented investigations was not to confirm these findings, but to provide

insight into and further challenge the 'free drug hypothesis', on the basis of mechanism-based PKPD modelling.

Mechanism-based modelling of β-blockers

The main objective of the research described in **section II** was the investigation of the influence of plasma protein binding on the PD of β-blockers by means of mechanism-based PKPD modelling. For the development of mechanism-based PKPD models a continuous measure of drug effect is required (i.e. heart rate, EEG effect, body temperature) (Dingemanse *et al*., 1988). To expand the dynamic range of this PD endpoint, the use of a continuous intravenous infusion of isoprenaline (5 $\mu g\ kg^{-1}\ h^{-1}$) in the PD was evaluated in **chapter 3**. To this end the effect of S(-) atenolol on heart rate in male Wistar Kyoto (WKY) rats was quantified with and without isoprenaline-induced tachycardia by means of PKPD modelling. In addition to the validation of the effect on isoprenaline-induced tachycardia as a PD endpoint, the concentration-effect relationship of isoprenaline was also characterised in WKY rats.

In **chapter 4**, a mechanism-based PD interaction model for the interaction between an antagonist and an agonists is proposed, which can be used for the estimation of *in vivo* affinity of β-blockers in presence of the agonist isoprenaline. As the first step in the model development, the concentration-effect relationship of isoprenaline was characterised using the operational model of agonism. This is important since this enables estimation of both the *in vivo* operational affinity and intrinsic efficacy of isoprenaline. This information is essential in the modelling of the interaction of interaction with the β-blockers. Subsequently, the interaction model was applied to the heart-rate profiles obtained in rats after administration of S(-)-atenolol under isoprenaline-induced tachycardia. In this manner, the *in vivo* affinity of S(-)-atenolol for the β-adrenoceptor could be obtained which was highly comparable to the known *in vitro* affinity for this compound.

Subsequently, the role of plasma protein binding on the PD of four β-blockers (atenolol, metoprolol, propranolol and timolol) with a varying extent of plasma protein binding was determined in comparative PKPD studies in conscious rats. In **chapter 5**, the previously developed mechanism-based interaction model (**chapter 4**) was used to characterise the PD interaction between isoprenaline and the individual β-blockers. The concentration vs. heart rate profiles were described on basis of both total and free drug concentrations and this yielded estimates of the *in vivo* affinity ($K_{B,vivo}$), which were then compared to the *in vitro* affinity ($K_{B,vitro}$) for the β-blockers. The results of this analysis provided clear indications that the free fraction in plasma is the main determinant of drug effect *in vivo* for the β-blockers.

Challenging the 'free drug hypothesis

Section III focuses on the investigation of the influence of plasma protein binding under conditions of altered plasma protein binding. For the purpose of investigations on the role of free drug concentrations as a determinant of the PKPD relationship of highly protein bound drugs, it is important to modulate the free fraction *in vivo* in a controlled manner. In **chapter 6**, a method was developed which modified plasma protein binding of S(-)-propranolol in rats in a robust, reproducible and time-dependent manner. Surgical implantation of permanent cannulas induced a ten- to fifteen fold increase in serum AGP concentrations at 2 days post-surgery, with return of the AGP levels back to baseline within one week. The altered AGP levels *in vivo* resulted in a clear and significant change in plasma protein binding of S(-)-propranolol. Subsequently it was confirmed in *ex-vivo* experiments that an increase in AGP serum concentration resulted in a profound decrease in the free fraction of S(-)-propranolol.

The main objective of the investigations described in **chapter 7** was to challenge the 'free drug

hypothesis', using the newly developed rat model for altered plasma protein binding (**chapter 6**). The influence of altered serum AGP levels on heart rate effects of S(-)-propanolol was studied by comparison of PKPD correlations in rats at 2 and at 7 days post surgery, with elevated and normal serum levels of AGP respectively. For S(-)-propranolol the $K_{B,vivo}$ decreased with increasing AGP concentrations in accordance to the "free drug hypothesis" and these results confirmed the findings of **chapter 5**.
Finally, in **Section IV**, **chapter 8**, the results of the investigations are discussed and perspectives for future research are presented.

1.3 References

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