The 'free drug hypothesis' : fact or fiction?

Steeg, T.J. van

Chapter 7

Challenging the 'free drug hypothesis': mechanism-based PKPD modelling of altered AGP binding on heart rate effects of S(-)-propanolol in rats

TJ van Steeg[1], EHJ Krekels[1], J Freijer[2], M Danhof[1] and ECM de Lange[1,3]

[1]Division of Pharmacology, LACDR, Leiden University, Leiden, Leiden, The Netherlands
[2]LAP&P Consultants BV, Leiden, The Netherlands
[3]Brains Online, Groningen, The Netherlands

Summary

Background and purpose The 'free drug hypothesis' was challenged by changing serum AGP levels and investigate the effect on heart rate of S(-)-propanolol by mechanism-based pharmacokinetic-pharmacodynamic (PKPD) modelling, using the operational model of agonism for estimation *in vivo* receptor affinity ($K_{B,vivo}$) .
Methods PKPD experiments were performed in male Wistar Kyoto rats on day 2 (elevated protein binding; n=7) and day 7 (normal protein binding; n=8) post surgery. Serial blood samples were taken to characterise the PK for S(-)-propranolol (i.v. infusion 20 μl min^{-1} for 15 min). Heart rate under isoprenaline-induced tachycardia (continuous i.v. infusion of 5 μg kg^{-1} h^{-1}) was used as a PD endpoint.
Results The plasma concentrations of AGP were 708 ± 274 *versus* 176 ± 110 $\mu g.ml^{-1}$ (mean ± SE) at day 2 and day 7 post surgery, respectively. A three compartment model described the PK of S(-)-propranolol best. The AGP concentration was a covariate for the inter-compartmental clearance for the third compartment. A log-linear relation was found between the plasma AGP concentration and $K_{B,vivo}$ based on total plasma concentrations. An increase in serum AGP concentration from 110 to 1150 μg mL^{-1} changed $K_{B,\ vivo}$ of total plasma concentrations of S(-)-propranolol from 4.6 nM to 30 nM.
Conclusions The new mechanistic PKPD interaction model is the first able to provide $K_{B,vivo}$ estimates for drugs with a target within the system circulation. For S(-)-propranolol, tested as first drug, the $K_{B,\ vivo}$ decreased with increasing AGP concentrations in accordance to the "free drug hypothesis".

7.1 Introduction

Plasma protein binding may affect both the pharmacokinetics (PK) and the pharmacodynamics (PD) of drugs, (Wright *et al.,* 1996; Pacifici and Viani, 1992; Colmenarejo, 2003; Bergogne-Berezin, 2002). At present the theoretical basis of the influence of (alterations in) plasma protein binding on PK is well-established (Rowland and Tozer, 1995). On the other hand, the impact of PPB on the PD has not been investigated in a quantitative manner. Available experimental evidence seems to indicate that for certain drugs (i.e. benzodiazepines, opiates, steroids) indeed the free concentration determines the intensity of the response (Cox *et al*., 1998; Derendorf *et al*., 1993; Mandema *et al*., 1991; Visser *et al*., 2003) and this has led to the practice of using the free fraction in plasma as a selection criterion in drug development (Trainor, 2007). However at present there is still limited experimental evidence for the so-called 'free drug hypothesis', which states that the intensity of the drug effect is related to the free rather than the total drug concentration. In this respect it is of interest that for certain drugs (i.e. A_1 adenosine agonists) experimental evidence seems to indicate that the opposite may be the case in the sense that it appears to be the total rather than the free drug concentration that determines the response (Van Der Graaf *et al*., 1997). Against this background we have recently started a series of investigations on β-adrenoreceptor agonists to study in detail the influence of plasma protein binding on pharmacodynamics using an integrated pharmacokinetic-pharmacodynamic (PKPD) approach (Van Steeg *et al*., 2007; 2008; chapter 5).
To answer the question whether the free or the total drug is the main determinant of drug effect mechanism-based PKPD modelling is uniquely suited, since by incorporation of concepts from receptor theory it enables estimation of the *in vivo* target affinity and intrinsic efficacy (Breimer and Danhof, 1997; Van Der Graaf and Danhof, 1997; Van Der Graaf *et al*., 1997; Danhof *et al*., 2007; Danhof *et al*., 2008). In a recent study, we have shown that that a meaningful estimate of the *in vivo* affinity of S(-)-atenolol for

the β-adrenoceptor could be obtained by analysis of the interaction with isoprenaline using a mechanism-based modelling approach based on receptor theory (van Steeg *et al*., 2008). Subsequently, we have examined the PKPD correlation of a series of β-blockers with widely different plasma protein binding, using heart-rate under isoprenaline-induced tachycardia as a PD endpoint. By analysis of the *in vivo - in vitro* correlation of the binding affinities evidence was obtained that it is indeed the free rather than the total drug concentration which determines the effect on heart rate for the β-blockers (chapter 5).
In the current investigation we have challenged the 'free drug hypothesis'. To this end the influence of altered PPB on the PKPD correlation of the highly protein-bound β-blocker S(-)-propranolol was determined *in vivo*.
To investigate the influence of altered plasma protein binding on PD, it is important to modulate the free fraction *in vivo* in a controlled manner. In previous work we have shown that surgical implantation of permanent cannulas induces a ten- to fifteen fold increase in serum AGP concentrations at 2 days post-surgery which normalizes within approximately one week (van Steeg *et al*., 2007b). Specifically, at 48 hours post surgery the AGP concentration was 1540 ± 122 $\mu g.ml^{-1}$ and the elevated AGP levels returned back to baseline (85 ± 21 $\mu g.ml^{-1}$) within one week. In addition it was shown that an increase in plasma AGP concentration from 55 to 675 $\mu g.ml^{-1}$ results in a decrease in the free fraction of S(-)-propranol from 14 ± 0.6 to 1.9 ±0.3%. Investigations on the PKPD correlation of propranolol at 2 and 7 days post surgery, therefore, enable examination of the influence of altered plasma protein binding on the pharmacodynamics.
The heart rate profiles were analysed using the previously developed PD interaction model to provide *in vivo* estimates of affinity ($K_{B,\ vivo}$) for S(-)-propranolol, for normal and for elevated plasma protein binding (van Steeg *et al*., 2008). If the "free drug hypothesis" would be applicable, higher plasma AGP levels would yield higher values of the *in vivo* binding constant $K_{B,vivo}$ based on total plasma concentrations, while the value of the affinity constant based on free drug concentrations should remain essentially unaltered.

7.2 Methods

Animals
All animal procedures were performed in accordance with Dutch laws on animal experimentation. The study protocol was approved by the Animal Ethics Committee of Leiden University (UDEC no. 04095). Male Wistar Kyoto rats (314 g ± 54, n=15) obtained from Janvier (Le Genest Saint Isle, France) were housed individually at a constant temperature of 21ºC and a 12-hour light/dark cycle. Prior to the surgery the rats were acclimatised for at least 5 days. The rats had *ad libitum* access to acidified water and food (laboratory chow, Hope Farms, Woerden, The Netherlands), except during the experimental procedures.

Drugs and Chemicals
S(-)-propranolol, metoprolol and (-)-isoprenaline hydrochoride (isoprenaline) were purchased from Sigma-Aldrich BV (Zwijndrecht, the Netherlands). Ketanest-S® ((S)-ketaminebase) was purchased from Parke-Davis (Hoofddorp, The Netherlands). Domitor® (medetomidine hydrochloride) was obtained from Pfizer (Capelle a/d IJssel, The Netherlands). Polyvinylpyrrolidone (PVP) was obtained from Brocacef (Maarsen, The Netherlands). Heparin (20 IU/ml) was obtained from the LUMC (Leiden University Medical Center) Pharmacy (Leiden, The Netherlands) and 0.9% (g/v) saline from B. Braun Melsungen AG. (Melsungen, Germany).

Surgery

The rats were anesthetised with a subcutaneous injection of 0.1 ml $100g^{-1}$ Ketanest-S® and an intramuscular injection of 0.01 ml $100g^{-1}$ Domitor®. During surgery the rats were placed on a heating pad to maintain body temperature at 37 °C. Two or seven days *prior* to the experiment, the rats were instrumented with four indwelling blood cannulas (Portex Limited, Hythe, Kent, England); two cannulas in the right jugular vein (Polythene 14 cm, ID 0.58 mm, OD 0.96 mm) for drug administration and one in the left and the right femoral artery (Polythene, 4 cm ID 0.28 mm, OD 0.61 mm + 20 cm ID 0.58 mm, OD 0.96 mm) for blood sampling and heart rate measurements respectively. The blood cannulas were subcutaneously tunnelled and externalised at the dorsal base of the neck. To prevent blood clotting, the arterial cannulas were filled with a 25% (w/v) PVP solution in a 0.9 % saline solution containing 20 IU/ml heparin. The venous cannula was filled with a saline solution containing 20 IU/ml heparin.

Experimental Design

Experiments were performed to characterise the PK and PD of S(-)-propranolol under elevated and normal plasma protein binding. The study was conducted according to a parallel group design in which rats were randomly divided into two groups. Previous work indicated that plasma protein binding of propranolol is elevated and normal on day 2 and day 7 post surgery, respectively (van Steeg *et al.*, 2007b). Therefore, the rats in both groups received 1 mg kg^{-1} S(-)-propranolol under isoprenaline-induced tachycardia on day 7 (n=7) or day 2 (n=8) post surgery.

S(-)-propranolol was dissolved in saline and administered as an intravenous infusion in 15 minutes (20 μl min^{-1}). Isoprenaline-induced tachycardia consisted of a continuous intravenous infusion of 5 μg kg^{-1} h^{-1} isoprenaline in 0.1% SMBS saline solution (van Steeg *et al.*, 2007a).

Serial arterial blood samples were collected in heparinised tubes at pre-defined time intervals (pre dose, 5, 14, 15, 16, 18, 20, 32, 36, 58, 66, 100, 134, 198 and 240 min post dose) for determination of S(-)-propranolol concentrations. At the start of the experiment, one sample was obtained for the quantification of the individual AGP concentration. Plasma samples were obtained by centrifugation (5 min; 5000 rpm) and stored at – 80°C until analysis. Heart rate was recorded continuously throughout the experiment as a PD endpoint.

Pharmacodynamic measurements

All experiments started between 8.00 and 9.00 AM to avoid influences of circadian rhythms. The baseline heart rate was recorded for 30 min, thereafter heart rate under isoprenaline-induced tachycardia was recorded for 30 min before commencing with the S(-)-propranolol infusion. At the end of each experiment, which was at approximately 240 min after the start of the S(-)-propranolol infusion, the continuous infusion of isoprenaline was stopped and heart rate was recorded for another 20 min. Arterial blood pressure and heart rate were measured from the cannulas in the femoral artery using a P10EZ-1 pressure transducer (Viggo-Spectramed BV, Bilthoven, The Netherlands), equipped with a plastic diaphragm dome (TA1017, Disposable Critiflo Dome, BD, Alphen a/d Rijn, The Netherlands). During the experiment the diaphragm dome was flushed with saline at a rate of 500 μl h^{-1} (Harvard 22-syringe pump, Harvard Apparatus Inc., South Natick, MA, USA). The pressure transducer was placed at the level of the heart of the rats, when in normal position, and connected to a blood pressure amplifier (AP-641G, Nihon Kodhen Corporation., Tokyo, Japan). Heart rate was captured from the pressure signal. The signals were passed through a CED 1401*plus* interface (Cambridge, Electronic Design LTD, Cambridge, England) into a Pentium 4 computer using the data acquisition program Spike 2 (Spike 2 Software, version 3.11, Cambridge, England) and

stored on a hard disk for off-line analysis.

Drug analysis
S(-)-propranolol concentrations were quantified using chiral reversed phase HPLC following liquid-liquid extraction as briefly described below. The HPLC-system consisted a LC-10AD HPLC pump (Shimadzu, 's Hertogenbosch, The Netherlands), a Waters 717 plus autosampler (Waters, Etten-Leur, The Netherlands), and a FP 920 fluorescence detector (Jasco Co, Tokyo, Japan) with an excitation wavelength of 230 nm and an emission wavelength of 340 nm. Chromatography was performed on a chiracel OD-R column (4.6 mm I.D. x 250 mm) (Diacel chemical industries LTD, Breda, The Netherlands) equipped with a commercially available guard column. The mobile phase consisted of 50% (v/v) 0.5 M sodium perchlorate aqueous solution and 50% (v/v) acetonitrile. Sample (50 μl plasma), internal standard (50 μl sotalol 2 μg/ml in water), sodium hydroxide solution (3 M, 100 μl), water (250 μl) and ethyl acetate (5 ml) were mixed, shaken (3 min) and centrifuged (4000 rpm, 10 min). The organic layer was taken and evaporated to dryness under vacuum (37 °C). Subsequently the residue was reconstituted in 100 μl mobile phase and 50 μl was injected into the HPLC-system. Linear calibration curves were obtained in the range 5-1000 ng ml^{-1} ($r>0.995$, $n=8$) and the limit of quantification for S(-)-propranolol and R(+)-propranolol were 10 and 5 ng ml-1, respectively.

AGP quantification
AGP concentrations were determined by a single radial immunodiffusion assay using a commercially available kit (Tridelta Development Ltd., Wichlow, Ireland). In short, 5 μl serum or standard solution was added to a well in an agar gel plate containing antibodies against (rat specific) AGP. The plate was placed in a humidified chamber of 37 °C for approximately 24 hours after which the AGP concentration was determined.

Data analysis
The previously developed PK model for isoprenaline served as an input for the data-analysis in this study (van Steeg *et al.*, 2007a). The PKPD relationship of isoprenaline was analysed previously on basis of the operational model of agonism and the results of this analysis were used in the current analysis as well (van Steeg *et al.*, 2008). The PK of S(-)-propranolol and the PD interaction between S(-)-propranolol and isoprenaline were quantified using non-lineair mixed-effects modeling as implemented in NONMEM software version V, level 1.1 (Beal and Sheiner, 1999). The approach takes into account structural effects and both intra- and interanimal variability. Parameters were estimated using the first-order conditional estimation method with η-ε interaction (FOCE interaction). Modelling was performed on an IBM-compatible computer (Pentium IV, 1500 MHz) running under Windows XP with the Fortran compiler Compaq Visual Fortran version 6.1. An in-house available S-PLUS 6.0 (Insightful Corp., Seattle, WA, USA) interface to NONMEM version V was used for data processing and management and graphical data display. Model selection was based on the objective function and the Akaike Information Criterion (AIC) (Akaike, 1974). Goodness-of-fit was determined by visual inspection of several diagnostic plots.

Pharmacokinetics PK analysis for S(-)-propranolol was performed by fitting a standard three compartment model to the concentration-time profile. Interanimal variability of the PK parameters was described according to an exponential distribution model:

$$P_i = \theta \cdot \exp(\eta_i) \quad (1)$$

in which P_i is the individual value of model parameter *P*, θ is the typical value (population value) of parameter *P* and η_i is the random deviation of P_i from *P*. The values of η_i are assumed to be independently normally distributed with mean zero and variance ω^2. Selection of an appropriate residual error model was based on inspection of goodness-of-fit plots. On this basis, a proportional error model was selected to describe residual error in the plasma drug concentration:

$$C_{obs,ij} = C_{pred,ij} \cdot (1 + \varepsilon_{ij}) \quad (2)$$

in which $C_{obs,ij}$ is the *j*th observed concentration in the *i*th individual, $C_{pred,ij}$ is the predicted concentration, and ε_{ij} accounts for the residual deviation of the model predicted value from the observed value. The values for ε_{ij} are assumed to be independently normally distributed with mean zero and variance σ^2. For S(-)-propranolol the individual PK parameter estimates served as an input for the PD model.

Pharmacodynamics A mechanism-based PD interaction model for isoprenaline and S(-)-propranolol was used to describe the heart rate response in rats. The mechanism-based model was applied under the assumption that the maximal obtainable effect in the system equals the maximal effect of isoprenaline (Kenakin, 1993;Black and Leff, 1983). An estimate for the *in vitro* affinity of isoprenaline was obtained from literature (Doggrell *et al*., 1998) and the efficacy (τ) of isoprenaline was estimated previously (van Steeg *et al*., 2008). The data obtained in the both treatment groups were analysed simultaneously using equation 3.

$$E = E_0 + \frac{E_{\max} \cdot (\tau \cdot [A])^n}{(K_A + [A] + ([B] \cdot \frac{K_A}{K_B}))^n + (\tau \cdot [A])^n} \quad (3)$$

In which *E* is the overall effect of the agonist at concentration *[A]* and the antagonist at the concentration *[B]*, E_0 is baseline heart rate, E_{max} is the maximum effect of the agonist, K_A and τ are the affinity and efficacy of the agonist respectively, K_B is the receptor affinity of the antagonist and *n* is the slope factor. At the start of the experiment, the observed baseline heart rate is under influence of the endogenous agonist, adrenalin. As a consequence, upon the administration of propranolol the heart rate was found to drop to a value below the original baseline. This can presumably be explained by the fact that the original baseline is under the influence of endogenous (nor)adrenalin. This was implemented in the model in a descriptive manner, since actual concentration-effect data on adrenalin and its interaction with S(-)-propranolol are not readily obtainable. In this analysis it was assumed that the baseline in the first 20 min (baseline) of the experiment is higher following equation 4.

$$E_0 = Base + DB \quad (4)$$

in which E_0 is the baseline heart rate in equation 3, *Base* is the unique baseline heart rate in the absence of endogenous adrenalin and *DB* is the factor describing the difference between the observed baseline and the true baseline heart rate. The estimated E_{max} for Isoprenaline ($E_{max\text{-}iso}$) is also under influence of this factor and, therefore, E_{max} was defined as follows (equation 5):

$$E_{max} = E_{max-iso} + DB \quad (5)$$

Interanimal variability of the PD parameters was described according to an additive (equation 6) or an exponential (equation 7) distribution model:

$$P_i = \theta + \eta_i \quad (6)$$

$$P_i = \theta \cdot \exp(\eta_i) \quad (7)$$

in which P_i is the individual value of model parameter P, θ is the typical value (population value) of parameter P and η_i is the random deviation of P_i from P. The values of η_i are assumed to be independently normally distributed with mean zero and variance ω^2.
On this basis of visual inspection, an additive error model was proposed to describe residual error in the drug effect:

$$C_{obs,ij} = C_{pred,ij} + \varepsilon_{ij} \quad (8)$$

in which $C_{obs,ij}$ is the jth observed concentration in the ith individual, $C_{pred,ij}$ is the predicted concentration, and ε_{ij} accounts for the residual deviation of the model predicted value from the observed value. The values for ε_{ij} are assumed to be independently normally distributed with mean zero and variance σ^2.

7.3 Results

In two parallel groups of rats, the mean AGP concentration at 2 days (n=7) and 7 days (n=8) post surgery was 708 ± 274 μg ml^{-1} and 176 ± 111 μg ml^{-1}, (mean ± SE) respectively, while the individual values ranged between 110 – 1150 μg ml^{-1} (Figure 3).

Pharmacokinetics A three compartment model adequately described the PK of S(-)-propranolol on day 2 (Figure 1) and day 7 post surgery (Figure 2). All PK parameters were estimated with good precision (CV<50%), except for the third volume of distribution (*V3*) with a coefficient of variation of 53% (Table I). Interanimal variability was identified for clearance (*Cl*) and the second volume of distribution (*V2*). Correlations between the values of interanimal variability were evaluated by using a full omega matrix. A significant correlation was obtained for *Cl* and *V2* and this correlation was taken into account in the final model by means of estimation of the covariance between both parameters. In addition, a significant correlation was identified between the AGP concentration and the intercompartmental clearance (*Q3*) for the third compartment (Figure 3). The AGP level was, therefore, included in the model as a covariate for *Q3*.

Pharmacodynamics A steep increase in heart rate was observed directly after the start of the intravenous isoprenaline infusion. Thereafter upon S(-)-propranolol administration the heart rate decreased again and slowly returned as S(-)-propranolol is cleared from the system. Finally, after the stop of the infusion of isoprenaline the heart rate decreased back to baseline.

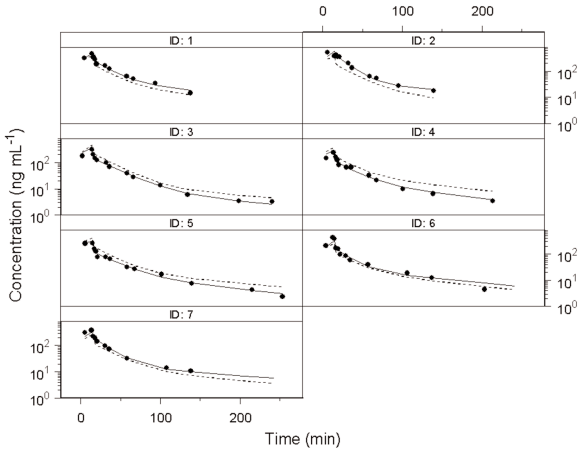

Figure 1, Individual plots final PK model for S(-)-propranolol two days post surgery (elevated plasma protein binding). The solid and broken lines represent the individual and the populations prediction, respectively. The dots represent the observed plasma concentrations for S(-)-propranolol.

In earlier studies, the concentration-effect relationship for isoprenaline has been analysed and the pharmacological effect on heart rate following isoprenaline and S(-)-propranolol administration was adequately described using the previously developed mechanism-based PD interaction model (van Steeg *et al*., 2007a;van Steeg *et al*., 2008). In this study both groups, with normal and elevated protein binding, were analysed simultaneously in three subsequent steps. First, the PD analysis was performed without implementation of a covariate effect for protein binding (on the basis of the serum AGP concentration). In this step, interanimal variability was identified for baseline (E_0), for the decreased baseline (*DB*) and for the receptor affinity constant (K_B). A large interanimal variability was observed for K_B. Due to the large variability, the individual estimates for K_B on day 2 (elevated AGP levels) were not found to be significantly greater (p=0.25) than on day 7 (normal AGP levels) using a standard two-sample t-test (figure 5A). Subsequently, treatment group (day 2 vs. day 7) was implemented as a categorical

Table 1, Population PK model for S(-)-propranolol: Estimates and coefficient of variation (CV%) for PK parameters, interanimal variability (ω) and residual error (σ).

Parameter	Value	CV (%)
Structural parameters		
CL (ml min^{-1})	28.5	12.0
V1 (ml)	89.0	26.0
Q2 (ml min^{-1})	62.5	14.1
V2 (ml)	556	17.4
Q3 (ml min^{-1})[a]	13.1	13.9
V3 (ml)	1160	53.7
Covariate AGP[b]	0.000676	13.7
Inter-animal variability		
ω_{CL}^2	0.101	33.5
ω_{V2}^2	0.174	44.7
ω_{CLxV2}^2	0.120	28.7
Residual error		
σ_{PK}^2	0.0353	14.6

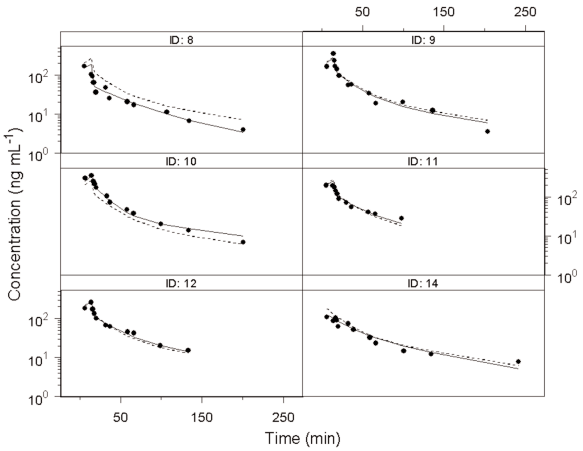

Figure 2, Individual plots final PK model for S(-)-propranolol seven days post surgery (normal protein binding). The solid and broken lines represent the individual and the populations prediction, respectively. The dots represent the observed plasma concentrations for S(-)-propranolol.

covariate in the PD model. Finally, the AGP concentrations were used as a continuous covariate for PPB. In this analysis a positive correlation was observed between the AGP concentration and the estimated affinity constant K_B and this covariate effect was included as a linear relationship between the AGP concentration and log K_B (figure 5B). A 10-fold increased serum AGP concentration (110 to 1150 μg/mL) changed the estimate for $K_{B,\ vivo}$ on the basis of total plasma concentrations of S(-)-propranolol from 4.6 nM to 30 nM. The model including AGP as covariate for K_B described the observed heart rate profiles best as judged on the objective function and the individual plots are shown in figure 4. All parameters were estimated with acceptable precision and the coefficients of variation ranged between 4.4 and 69% (Table 2). Residual variability was described using an additive error model and was not found to be significantly different between both treatment groups.

7.4 Discussion and conclusion

The objective of the current study was to challenge the free drug hypothesis by investigating the influence of altered plasma protein binding on the pharmacodynamic of propranolol in rats. In this study we used a newly developed rat model for *in vivo* mechanism-based population PKPD modelling enabling estimation of *in vivo* receptor affinities ($K_{B,vivo}$) (van Steeg *et al.*, 2008). 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 plasma levels of AGP respectively. AGP was found to be a statistically significant covariate of the estimates of $K_{B,vivo}$ based on total plasma concentrations. The log $K_{B,vivo}$ values were found to increase in a log linear manner with increasing AGP concentrations, indicating that plasma protein binding restricts the drug effect.

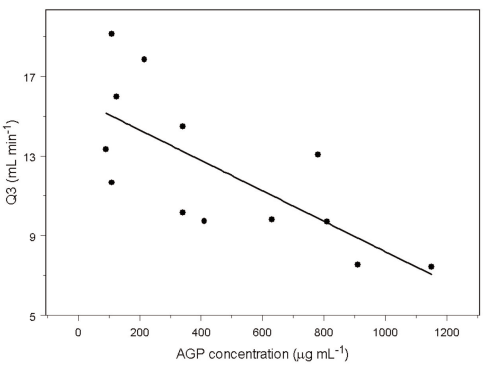

Figure 3, Correlation between AGP concentration and intercompartmental clearance for the 3rd compartment (Q3) (r^2=0.53). The correlation is shown for the PK model without the covariate AGP for Q3 included.

To determine the influence of altered plasma PPB of

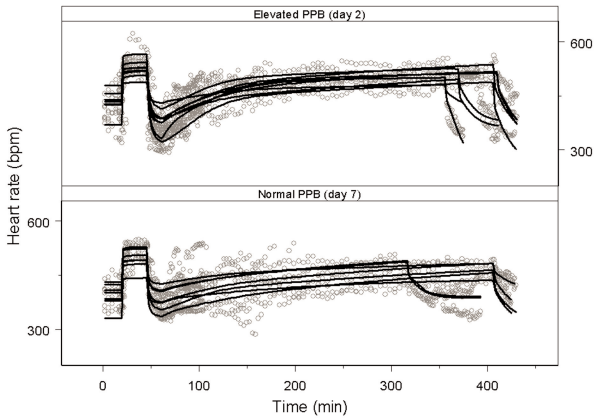

Figure 4, Individual plots for the model fit of the PD after administration of S(-)-propranolol under isoprenaline-induced tachycardia. The solid lines indicate the individual prediction of the model. The grey open symbols represent the observations.

a drug *in vivo* it is important to use an integrated PKPD approach to account for simultaneous changes in pharmacokinetics. Moreover it is important to obtain estimates of the *in vivo* receptor affinity ($K_{B,vivo}$). In general, the estimation of $K_{B,vivo}$ on basis of PD endpoints (drug effects) is difficult and requires extensive knowledge of the system under investigation. In this respect it is important that estimates of the potency of a drug obtained on the basis of descriptive models such as the Hill equation depend on both the receptor affinity and intrinsic efficacy of the drugs under investigation. Moreover the degree of receptor expression (i.e. receptor reserve) and system-specific transduction functions are additional determinant of *in vivo* concentration-effect relationships. For this concepts from receptor theory are increasingly applied in mechanism-based PKPD modeling for estimation of the *in vivo* operational affinity and intrinsic efficacy of drugs (Van der Graaf and Danhof, 1997; Van der Graaf *et al.,* 1997; Danhof *et al*., 2007; 2008). Recently we have developed a mechanism-based population modelling approach to obtain a meaningful estimate of $K_{B,vivo}$ taking into account the endogenous agonist (adrenalin), the applied agonist (isoprenaline) and the antagonist (β-blocker) (van Steeg *et al*., 2008). As a consequense, this model constitutes a useful basis for investigation of the role of plasma protein binding on the pharmacodynamics of β-blockers as model drugs.

In a previous investigation we have investigated the role of plasma-protein binding on the pharmacodynamics by mechanism-based PKPD modelling of the effects of 4 different β-blockers with

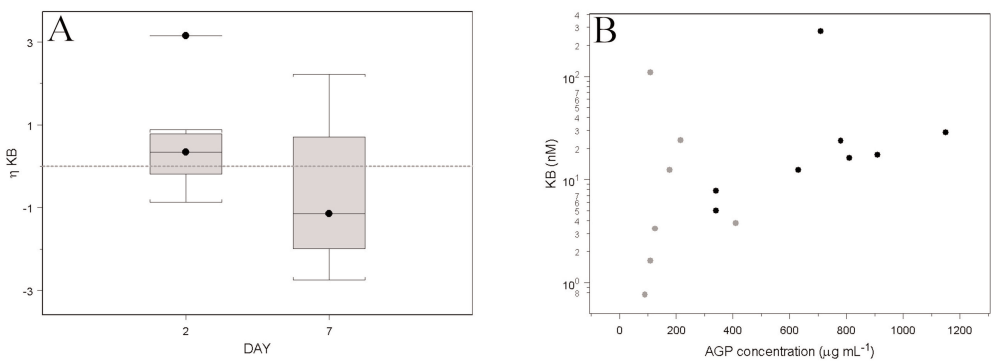

Figure 5, A. Interindividual variability in the estimate for K_B plotted against day post surgery. B. The estimate for K_B plotted against the AGP concentration in serum (r^2=0.20). The grey and black dots represent the observations at day 7 and 2 respectively.

widely different plasma protein binding. On the basis of the analysis of the correlation between the *in vivo* binding affinity $K_{B,vivo}$ and the corresponding *in vitro* binding affinity evidence was obtained that indeed de free drug concentration is the determinant of the drug effect (chapter 5).

In the current investigation the previous conclusion was challenged by studying the PKPD of S(-)-propranolol under conditions of altered PPB resulting from different levels of plasma AGP concentrations, at 2 and 7 days post surgery (Van Steeg *et al*., 2007a; 2007b). This analysis revealed that AGP concentration is a significant covariate of both the pharmacokinetics and the pharmacodynamics. The PK of S(-)-propranolol was slightly but significantly influenced by the changes in the concentration of AGP. Specifically, the AGP concentrations were inversely related to the individual estimates for the intercompartmental clearance (Q3) for the third compartment (figure 4). Estimation of the AGP levels as a covariate for Q3 resulted in a significant improvement of the PK model. It can, therefore, be concluded that the increase in PPB restricts the distribution to the third compartment. In this study, the changes in PPB did not affect the clearance of the drug significantly. This is in agreement with findings by Terao *et al.,* who reported only minor changes in the PK of l-propranolol after an i.v. administration 2 and 8 days post catheter implantation (Terao and Shen, 1983). In addition to the small reduction in volume of the central compartment in that study, a significant reduction in the terminal half-life was observed at 8 days post catheter implantation. Like in humans, propranolol is a high extraction drug in rats (E_H > 0.7) and clearance is, thus, nearly independent of the free fraction (Suzuki *et al*., 1980). This is in agreement with the current findings.

A number of other studies reported large and statistically significant changes in the PK of propranolol upon changes in plasma protein binding (i.e. reduction in intrinsic clearance, elevated AUC) (Yasuhara *et al*., 1985; Chindavijak *et al*., 1988; Belpaire and Bogaert, 1989). However, the observed differences are most likely related to factors other than changes in plasma protein binding, related to the fact that the studies were performed within 2 hours post catheter implantation.

Table 2, Population PD interaction model for isoprenaline and S(-)-propranolol: Estimates and coefficient of variation (CV%) for PD parameters, interanimal variability (ω) and residual error (σ).

Parameter	Value	CV (%)
Structural parameters		
E_0 *(bpm)*	349	4.4
D_B *(bpm)*	65.7	31.6
E_{max} *(bpm)*[a]	130	FIX
τ[f]	247	FIX
$K_A = K_i$ *(nM)*[b]	30.0	FIX
Log K_B *(nM)*	0.843	21.1
n	0.698	15.0
Ke0 (min^{-1})	0.202	18.4
Covariate AGP[c]	-9.27e-4	-68.9
Inter-animal variability		
ω_{E0}^2	2300	43.1
ω_{DB}^2	4400	34.1
Residual error		
σ_{PD}^2	1230	17.4

S(-)-propranolol is a β-adrenoceptor antagonist (β-blocker) has been used often in investigations concerning the influence of altered PPB on PK (Belpaire *et al*., 1986; Chindavijak *et al*., 1988; Terao and Shen, 1983). and on *in vivo* drug effects (Belpaire *et al*., 1986; Yasuhara *et al*., 1985). None of these studies, however, have used (PK-)PD modelling as a tool to discriminate between the influence of PPB on drug exposure and the influence on drug effect.

A considerable inter-individual variation in the AGP concentrations was observed in the animals of the two treatment groups. This complicated the statistical analysis of the AGP effect by a standard comparison of the parameter estimates between the two treatment groups (figure 5a). However incorporation of AGP as a covariate in the population PKPD analysis enabled the analysis of the relation between AGP concentration and the values of the receptor binding affinity constant $K_{B,in\ vivo}$. In this respect it is on interest that there is an almost continuous distribution of the AGP concentration with values ranging from 110-1150 μg ml^{-1}.

Although the estimation of the covariate was somewhat imprecise (CV = 69%) due to the large interanimal variability, inclusion of the covariate effect significantly improved the goodness of fit. PPB influences the PD of S(-)-propranolol. As a consequence, free drug concentrations are considered the best predictor of drug effect for S(-)-propranolol. This is confirmed by the observation that the estimate for affinity *in vivo* based on total drug (K_B =6.96 nM; 95% confidence interval 3.09-15.4 nM) is significantly different from the *in vitro* affinity (K_B = 1.91 ± 0.48 nM) (Chiu *et al*., 2004; Juberg *et al*., 1985; Louis *et al*., 1999; Nandakumar *et al*., 2005). The findings in the current study are, thus, in agreement with previous studies. Moreover the conclusions are consistent with our previous work on basis of a mechanism-based PKPD model a series of β-blockers (chapter 5).

The serum AGP concentration increased 10-fold from 110 (day 2) to 1150 μg/mL (day 7). On basis of previous investigations on the relation between AGP concentration and the free fraction of propranolol (Van Steeg *et al*., 2007b), the estimated free fractions of propranolol in the present investigation were 8.3% and 1.4% on day 2 and day 7 respectively (van Steeg *et al*., 2007b). The estimates for $K_{B,\ vivo}$ on the basis of total plasma concentrations of S(-)-propranolol were 4.6 nM to 30 nM under conditions of normal and elevated PPB respectively. Consequently, the corrected estimates for $K_{B,\ vivo}$ on basis of free drug concentrations were 0.38 nM and 0.41 nM. Correcting for plasma protein binding, thus, resulted in nearly identical estimates of affinity and this further strengthens the conclusion that it is the free rather than the total concentration is the main determinant of drug effect for S(-)-propranolol. In conclusion, the “free drug hypothesis” appears to be applicable to the PD of S(-)-propranolol.

In conclusion, the mechanism-based interaction model adequately described the effect on heart rate S(-)-propranolol under isoprenaline-induced tachycardia for both normal and elevated plasma protein binding. Moreover, it can be concluded that the “free drug hypothesis” does hold for the effect on heart rate of S(-)-propranolol.

In conclusion, using the previously newly developed mechanism-based PKPD interaction model we were able to adequately describe the effect of S(-)-propranolol on heart rate under isoprenaline-induced tachycardia for both normal and elevated PPB, and it was shown that the $K_{B,\ vivo}$ for S(-)-propranolol decreased with increasing AGP concentrations. This approach is generally applicable for the estimation of $K_{B,vivo}$ values for drugs with a target within the general circulation. While the “free drug hypothesis” does hold for the effect on heart rate of S(-)-propranolol, the hypothesis needs further testing to challenge its general applicability.

7.5 References

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