The 'free drug hypothesis' : fact or fiction?

Steeg, T.J. van

Chapter 3

Pharmacokinetic-Pharmacodynamic modelling of S(-)-Atenolol in rats: Reduction of Isoprenaline-Induced Tachycardia as a Continuous Pharmacodynamic Endpoint

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Summary

Background and purpose For the development of mechanism-based pharmacokinetic-pharmacodynamic (PKPD) models continuous recording of drug effects in PKPD studies is essential. We therefore explored the use of isoprenaline in the continuous measurement of the cardiovascular effects of β-blockers. The aim was to validate heart rate as a pharmacodynamic endpoint under continuous isoprenaline-induced tachycardia by means of PKPD modelling of S(-)-atenolol.
Methods An isoprenaline (n=9) and non-isoprenaline (n=8) group of WKY rats received a 15 min iv infusion of 5 mg kg^{-1} S(-)-atenolol, with or without iv infusion of 5 µg kg^{-1} h^{-1} isoprenaline respectively. Heart rate was continuously monitored and blood samples were taken.
Results A three-compartment model best described the pharmacokinetic data of S(-)-Atenolol. The PKPD relationship was described by a sigmoid Emax and an effect compartment was used to resolve the observed hysteresis. For the non-isoprenaline group the variability in heart rate (30 bpm) approximated the maximal effect (E_{max} = 43 ± 18 bpm), leaving the parameter estimate of potency (EC_{50} = 28 ± 27 ng ml^{-1}) unreliable. Both precise and reliable parameter estimates were obtained under isoprenaline-induced tachycardia: 517 ± 13 bpm (E_0), 168 ± 15 bpm (E_{max}), 49 ± 14 ng ml^{-1} (EC_{50}), 0.042 ± 0.012 min^{-1} (k_{eo}) and 0.95 ± 0.34 (n).
Conclusions Reduction of heart rate under isoprenaline-induced tachycardia is a reliable pharmacodynamic endpoint for β-blockers *in vivo* in rats. Consequently this experimental approach will be used to investigate the relationship between β-blocker drug characteristics and their *in vivo* effects.

3.1 Introduction

Mechanism-based pharmacodynamic modelling represents a specific area of pharmacokinetic-pharmacodynamic (PKPD) modelling in which specific drug related properties are linked to the *in vivo* concentration-effect relationship in order to characterise and predict the time course of drug effects *in vivo* (Black and Leff, 1983; Breimer and Danhof, 1997; Van Der Graaf and Danhof, 1997). It has been successfully applied to synthetic opiates, adenosine A_1 agonists and $5HT_{1A}$ agonists (Cox *et al*., 1998; Van Der Graaf *et al*., 1997; Van Der Graaf *et al*., 1999; Zuideveld *et al*., 2004). The possibility of a mechanism-based analysis is dependent on the pharmacological response measured. Though full concentration-time profiles are not always needed (Gabrielsson and Weiner, 1997; Gabrielsson *et al*., 2000), the pharmacological response should preferably be continuous, reproducible, objective, selective and sensitive for the system under investigation (Dingemanse *et al*., 1988). Moreover, for extrapolation of of preclinical data to drug response in humans and/or patients, the use of a common pharmacological response endpoint is preferable. Such endpoints include changes in the electroencephalogram, heart rate and body temperature.
β-adrenoceptors antagonists (β-blockers) are suitable model 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 potency for binding to β-adrenoceptors (Mehvar and Brocks, 2001; Riddell *et al*., 1987; Singh, 2005; Johnsson and Regardh, 1976).
The chronotropic effect of the β-blockers is mediated primarily by competition with endogenous agonists (noradrenalin) at the β_1-adrenoceptors, and is readily available as pharmacodynamic endpoint both in humans and in laboratory animals (Piercy, 1988; Wellstein *et al*., 1987; Kendall, 1997).

However, the reduction in heart rate after β-blocker administration is small and difficult to distinguish from normal variations in heart rate. In clinical investigations the pharmacological response of β-blockers is for that reason evaluated using isoprenaline-induced or exercise-induced tachycardia (Schafers *et al.*, 1999; Lipworth *et al.*, 1991; Van Bortel *et al.*, 1997). Isoprenaline-induced tachycardia is obtained by short infusions of isoprenaline and the effect isoprenaline on heart rate is evaluated with and without β-blocker being present. A comparable methodology is used for exercise-induced tachycardia, in which the responsiveness of heart rate to exercise is evaluated with and without β-blocker. In clincical studies, exercise-induced tachycardia is often preferred over isoprenaline-induced tachycardia because of safety issues and practical considerations.
The aim of this study is the validation of heart rate under continuous isoprenaline-induced tachycardia as a pharmacodynamic endpoint for β-blockers to be used in preclinical PKPD studies for the development of mechanism-based PKPD models. A secondary objective is establishment of a concentration-effect relationship for isoprenaline in Wistar Kyoto (WKY) rats. In the present investigation, a PKPD modelling approach is used for the validation, which is not mechanism-based.
The use of isoprenaline-induced tachycardia is evaluated since this is most easy to accomplish in the rat, as the increase in heart rate is controllable and can be attained continuously. Tachycardia was induced by a constant intravenous infusion of isoprenaline throughout the experiment. The β-blocker S(-)atenolol was used as a model compound, since it is a β_1-selective hydrophilic β-blocker without intrinsic sympathomimetic activity that is eliminated predominantly via the kidneys (Reiter, 2004; Kirch and Gorg, 1982). Furthermore, the active enantiomer of this drug, S(-)-atenolol is not metabolised into (inter)-active metabolites *in vivo* and has negligible protein binding (Reeves *et al.*, 1978a; Reeves *et al.*, 1978b).

3.2 Methods

Animals
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. 02112). Male Wistar Kyoto rats (294 g ± 49, n=39) 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(-)-Atenolol, Sotalol, (-)-isoprenaline hydrochoride (isoprenaline), 3,4-Dihydroxybenzylamine hydrobromide (DHBA), Sodium Metabisulfite (SMBS), diphenylboric acid 2-amino-ethanol ester (DPBEA) and tetraoctyl ammonium bromide (ToABr) 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^{-1}) was obtained from the LUMC (Leiden University Medical Center) Pharmacy (Leiden, The Netherlands) and 0.9% (g v^{-1}) saline from B. Braun Melsungen AG. (Melsungen, Germany). The DPBEA buffer consisted of a 2 M NH_4OH-NH_4CL buffer (pH 8.8) with 0.2% (w v^{-1}) DPBEA and 0.5% (w v^{-1}) EDTA. The heptane mixture consisted of *n*-heptane with 1% *n*-octanol and 0.25% (w v^{-1}) ToABr

Surgery

The rats were anesthetised with a subcutaneous injection of 0.1 ml 100 g^{-1} Ketanest-S® and an intramuscular injection of 0.01 ml 100 g^{-1} Domitor®. During surgery the rats were placed on a heating pad to maintain body temperature at 37 °C. 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 tunneled and externalised at the dorsal base of the neck. To prevent blood clotting, the arterial cannulas were filled with a 25% (w v^{-1}) PVP solution in a 0.9 % saline solution containing 20 IU ml^{-1} heparin. The venous cannula was filled with a saline solution containing 20 IU ml^{-1} heparin.

Experimental Design

The pharmacokinetic (PK) and pharmacodynamic (PD) experiments were performed in conscious WKY rats

S(-)-Atenolol The PK and PD of S(-)-atenolol (5 mg kg^{-1}) were determined in two groups. One group (isoprenaline; n=9) received an intravenous infusion of S(-)-atenolol (5 mg kg^{-1}; 15 min) with isoprenaline-induced tachycardia, the other group (non-isoprenaline; n=8) received an intravenous infusion of S(-)-atenolol without isoprenaline-induced tachycardia. Tachycardia was induced by means of a continuous intravenous infusion of 5 µg kg^{-1} h^{-1} of isoprenaline, since this dose provided an adequate effect-time profile with S(-)-atenolol. In the non-isoprenaline group the continuous infusion consisted of vehicle solution only (0.1% (w w^{-1}) SMBS-saline). The continuous infusion of isoprenaline or vehicle started at least 30 min before the start of the S(-)-atenolol infusion. S(-)-atenolol was dissolved in saline (0.9% (g v^{-1}) sodium chloride solution) and was administered intravenously during 15 min.

Serial arterial blood samples were collected in heparin tubes at pre-defined time intervals (0, 5, 10, 15, 17.5, 20, 22.5, 27.5, 32.5, 40, 55, 70, 90, 120, 180, 240, 360 and 480 min post-atenolol infusion) for determination of S(-)-atenolol concentrations. Plasma samples were obtained by centrifugation (5 min; 5000 rpm) and stored at –20°C until analysis. Heart rate was recorded continuously throughout the experiment.

Isoprenaline Isoprenaline is an extremely potent β-adrenoceptor agonist and a maximal increase in heart rate in rats is already obtained at plasma concentrations below the limit of quantification (LOQ). Therefore the PK and PD of isoprenaline were determined in separate experiments and the concentrations in the PD experiments were predicted using the PK model. For the PK, rats were randomly assigned to two treatment groups of 7 animals, which received either a intravenous infusion of 25 µg kg^{-1} or 50 µg kg^{-1} isoprenaline during 10 min. The doses used provide plasma concentrations, which are sufficiently above the LOQ. Similar to in the S(-)-atenolol experiments, isoprenaline was dissolved in a 01% (w w^{-1}) SMBS-saline solution for administration. Serial arterial blood samples were drawn for determination of isoprenaline concentrations. Because of the fast elimination of isoprenaline, the sampling schemes were slightly different in the two dose groups to ensure adequate estimation of the maximal concentration. For 25 µg kg^{-1}, blood samples were taken at 0, 2, 5, 8, 10, 11, 12, 13, 15, 17.5, 20, 25 and 30 min. For 50 µg kg^{-1}, blood samples were taken at at 0, 1, 3, 5, 9, 10.5, 11, 12, 13, 17.5, 20, 25 and 30 min. In total 14 serial blood samples were collected in heparin tubes containing 0.1% SMBS. Plasma (50-150 µl) was obtained immediately by centrifugation (5 min; 5000 rpm) and samples were analysed on the same day as the experiment.

For the characterization of the PD, 6 rats received various continuous intravenous infusions of isoprenaline, to be precise 0.001, 0.01, 0.05, 0.1, 0.2, 0.5, 1, 2.5 μg kg^{-1} h^{-1}. Additional data from other experiments in which rats were given a steady state infusion of 5 (n=17) and 10 μg kg^{-1} h^{-1} (n=8) were also used in the analysis. Throughout the experiment heart rate was recorded continuously and was used as a PD endpoint.

Pharmacodynamic measurements
All experiments started between 8.00 and 9.00 AM to avoid influences of circadian rhythms. 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(-)-atenolol and isoprenaline were quantified using reversed phase high performance liquid chromatography (HPLC) following liquid-liquid extraction as briefly described below.

S(-)-atenolol 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 235 nm and an emission wavelength of 300 nm. Chromatography was performed on Spherisorb ODS-2 3 μm column (4.6 mm I.D. x 100 mm) (Waters, Millford, MA, USA) equipped with a refill guard column (2 mm I.D. x 20 mm) (Upchurch Scientific, Oak Harbor, WA, USA) packed with pellicular C18 (particle size 20-40 μm) (Alltech, Breda, The Netherlands). The mobile phase consisted of 77.5% (v v^{-1}) 0.1 M sodium acetate buffer (pH 3.4) containing 5 mM octane-sulfonic acid (OSA) and 22.5% (v v^{-1}) acetonitrile. Sample (50 μl plasma), internal standard (50 μl Sotalol 5 μg ml^{-1} in water), sodium hydroxide solution (3 M, 100 μl), water (200 μl) and ethyl acetate (5 ml) were mixed, shaken (5 min) and centrifuged (4000 rpm, 10 min). The organic layer was taken and evaporated to dryness. 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 40-20000 ng ml^{-1} ($r>0.995$, n=10) and the limit of quantification for S(-)-atenolol was 40 ng ml^{-1}. The intra- and inter-assay variabilities were 4 and 11% respectively.

Isoprenaline The HPLC-system consisted a LC-10AD HPLC pump (Shimadzu, 's Hertogenbosch, The Netherlands), a Waters 717 plus autosampler (Waters, Etten-Leur, The Netherlands), a pulse damper (Antec Leyden, Zoeterwoude, The Netherlands) and a digital electrochemical amperometric detector (DECADE, software version 3.02, Antec Leyden, The Netherlands). The optimal working potential for (-)-isoproterenol was +0.65 V. Chromatography was performed on Ultraphere® C_{18} 5 μm column (4.6 mm I.D. x 150 mm) (Alltech, Breda, The Netherlands) equipped with a refill guard column (2 mm I.D. x 20 mm)

(Upchurch Scientific, Oak Harbor, WA, USA) packed with pellicular C18 (particle size 20-40 µm) (Alltech, Breda, The Netherlands) at a constant temperature of 30 °C.
The analysis was preceded by an ion-paired liquid-liquid extraction procedure, in which DPBEA and ToABr were used as complexing agents (Smedes *et al*., 1982). In short, Sample (50 µl plasma), internal standard (50 µl DHBA 1 µg ml^{-1} in 0.05 M citric acid solution), DPBEA buffer (250 µl pH= 8.8), water (500 µl) and heptane mixture (1.5 ml) were mixed, shaken (3 min) and centrifuged (4000 rpm, 8 min). The organic layer was transferred to clean tubes and 2 ml n-octanolol was added. Subsequently the organic mixture was back-extracted with 100 µl phosphoric acid solution (87 mM). Finally 30 µl of the aqueous phase was injected into the HPLC-system. Linear calibration curves were obtained in the range 1 -1000 ng ml^{-1} (r>0.995, n=7, 50 µl plasma) and the limit of quantification for Isoprenaline was 0.3 ng ml^{-1} based on a sample of 150 µl plasma. The intra- and inter-assay variabilities were 5 and 12% respectively.

Data analysis
The PK and PD of S(-)-atenolol and isoprenaline were quantified using non-lineair mixed-effects modelling 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 inter-individual variability (IIV). 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. Goodness-of-fit was determined using the objective function and by visual inspection of the plots of individual predictions and the diagnostic plots of (weighted) residuals. For nested models, a decrease of 10.8 points in the objective function, corresponding to $p < 0.001$ in a χ^2-distibution, by adding an additional parameter was considered statistically significant. In case of a comparison of models, which are structurally different, the models were compared using the Akaike Information Criterion (Akaike, 1974).

Pharmacokinetics – The PK analysis for S(-)-atenolol was performed by fitting a standard three compartment model to the concentration-time profiles. Pharmacokinetic compartmental analysis for isoprenaline was performed by fitting a standard two compartment model to the concentration-time profiles. Interanimal variability (IIV) of the pharmacokinetic parameters was described according to an exponential distribution model:

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

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:

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

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 both S(-)-atenolol and isoprenaline, the pharmacokinetics served as an input for the pharmacological model.

Pharmacodynamics - The concentration-effect relationships of S(-)-atenolol and isoprenaline were described using a sigmoidal Emax model according to:

$$E = E_0 + \frac{E_{max} \cdot C^n}{EC_{50}^n + C^n} \quad (3)$$

where E is the effect of the drug at concentration C, E_0 is the no-drug response, E_{max} is the maximum drug effect, EC_{50} is the drug concentration at half-maximal effect and n is the slope factor, which determines the steepness to the curve (i.e. the Hill factor).
An effect compartment was used to resolve the observed hysteresis between the plasma concentration and the effect of S(-)-atenolol. The following differential equation can be used under the assumption that the effect site concentration equals the plasma concentration in equilibrium.

$$\frac{dC_e}{dt} = k_{eo} \cdot (C_p - C_e) \quad (4)$$

where C_p represents the plasma concentration, C_e represents the effect-site concentration and k_{eo} is the first order rate constant describing drug transport.
For this drug, the pharmacodynamic parameters (P) were modeled as follows:

$$P_i = \theta + \eta_i \quad (5)$$

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:

$$C_{obs,ij} = C_{pred,ij} + \varepsilon_{ij} \quad (6)$$

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 ω^2.

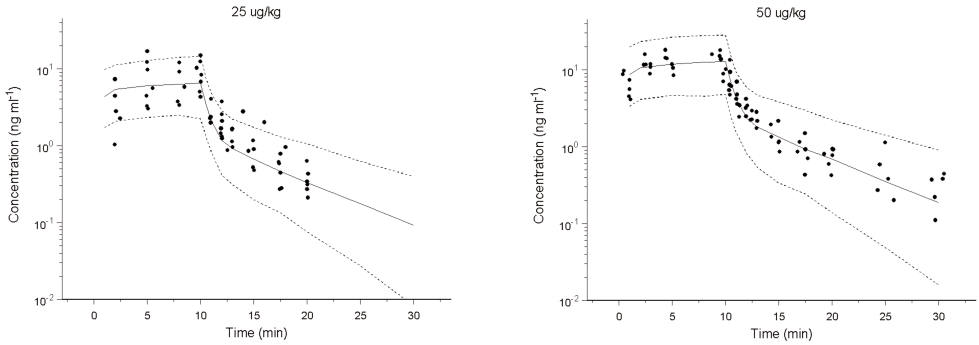

Figure 1, Visual predictive check of the population PK model for isoprenaline. The range between the dashed lines depicts the 90% inter-quantile range. The solid line presents the population prediction. The solid dots are the observed concentrations.

3.3 Results

Isoprenaline

Pharmacokinetics Based on visual inspection of the model fit and the objective function a two compartment model was selected for isoprenaline. This model adequately described the pharmacokinetics of isoprenaline with estimation of the rate constant of elimination (*k10*), the rate constant from central to peripheral compartment (*k12*), the rate constant from peripheral to central compartment (*k21*) and the volume of distribution (*V1*). All pharmacokinetic parameters were estimated precisely with acceptable coefficients of variance (Table 1). The coefficient of variation of the parameter estimates varied between 17 and 47%. Estimation of interindividual variability was possible for *k12*, *k21* and *V1*. To validate the pharmacokinetic model for isoprenaline, a bootstrap validation and a predictive check were performed. The population pharmacokinetic estimates were in good agreement with the estimates obtained by fitting 1000 data sets to the final population PK model (Table 1). The bootstrap revealed an

Table 1, Population estimates of PK parameters (micro constants) for isoprenaline in the rat and estimates of the bootstrap replicates.

Parameter	Value (CV%)	Value BS (CV%)
Structural parameters		
K10 (min^{-1})	1.32 (20.5%)	1.33 (21.5%)
K12 (min^{-1})	0.391 (36.1%)	0.412 (39.8%)
K21 (min^{-1})	0.177 (17.6%)	0.177 (20.2%)
V1 (ml)	79.6 (24.4%)	83.3 (26.1%)
Interindividual variability (IIV)		
ω_{K12}^2	0.213 (46.8%)	0.215 (54.1%)
ω_{K21}^2	0.114 (43.8%)	0.121 (103.0%)
ω_{V1}^2	0.0982 (47.1%)	0.0900 (49.5%)
Residual error		
σ_{Pk}^2	0.0796 (22.4%)	0.0760 (22.7%)

uncertainty in estimation of the interanimal variability on *k21* (CV=103%), though the estimate from the bootstrap replicates was nearly identical to the final population estimate. The predictive check showed that the PK model could well predict the time course of isoprenaline after intravenous infusion (Figure 1).

Pharmacodynamics - Isoprenaline is an extremely potent β-adrenoceptor agonist and a maximal increase in heart rate is already obtained at plasma concentrations below the detection limit in rats. As a result it is not possible to obtain a concentration-effect relationship in a single experiment. Plasma concentrations in the PD experiment were, therefore, extrapolated using the 2-compartment population PK model. Subsequently the concentration-effect relationship of isoprenaline was fitted to a sigmoidal Emax model. The following parameter estimates were obtained: 374.0 ± 7.0 beats min^{-1} (bpm) for baseline (E_0), 130 ± 7.7 bpm for maximal effect (E_{max}), 0.014 ± 0.0044 ng ml^{-1} for the concentration at half maximal effect (EC_{50}) and 1.18 ± 0.23 for the Hill coefficient (n). All PD parameters were estimated precisely with acceptable coefficients of variance and interanimal variability was observed for the baseline only (Table 2). The observed and population-predicted concentration-effect relationship of isoprenaline is displayed in figure 2.

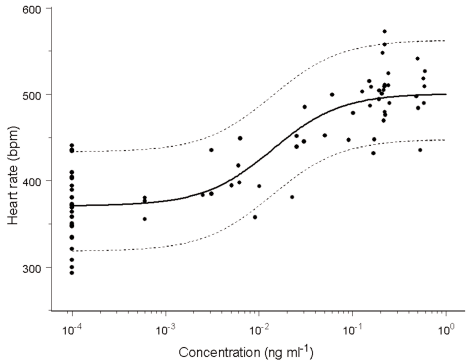

Figure 2, Population prediction (solid line) and observations (symbols) for the concentration-effect relationship for isoprenaline. The range between the dashed lines depicts the 90% inter-quantile range.

Table 2, Isoprenaline Population estimates of PD parameters and variabilities in the rat. E_0 represents the baseline heart rate, E_{max} the maximal effect of isoprenaline, EC_{50} the potency of the drug and n is the Hill coefficient.

Parameter	Value (CV%)
Structural parameters	
E_0 *(bpm)*	374 (1.9%)
E_{max} *(bpm)*	130 (5.9%)
EC_{50} *(ng* ml^{-1}*)*	0.0138 (31.9%)
n	1.18 (19.3%)
Interindividual variability (IIV)	
ω_{E0}^2	860 (29.8%)
Residual error	
σ_{PD}^2*(*bpm^2*)*	409 (18.3%)

S(-)-atenolol

Pharmacokinetics No distinct difference was observed between the isoprenaline and the non-isoprenaline group. Based on visual inspection of the plots and the objective function a three-compartment pharmacokinetic model was selected as PK model for both groups. All pharmacokinetic parameters were

Table 3, S(-)-atenolol population parameter estimates including variabilities and bootstrap replicates

Parameter	Value (CV%)	Value BS (CV%)
Structural parameters		
CL (ml min^{-1})	11.7 (3.4%)	11.7 (3.5%)
V1 (ml)	115 (7.4%)	114 (8.6%)
Q2 (ml min^{-1})	15.0 (7.2%)	15.2 (10.8%)
V2 (ml)	173 (7.7%)	172 (8.1%)
Q3 (ml min^{-1})	8.50 (5.3%)	8.50 (5.6%)
V3 (ml)	849 (4.2%)	848 (4.2%)
Interindividual variability (IIV)		
ω_{CL}^2	0.033 (9.8%)	0.032 (19.9%)
ω_{V2}^2	0.170 (28.2%)	0.169 (29.6%)
ω_{V3}^2	0.023 (36.7%)	0.022 37.5%)
$\omega_{CL,V3}^2$ *(covariance)*	0.026 (31.9%)	0.024 (30.8%)
Residual error		
σ_{PK}^2	0.027 (9.5%)	0.027 (9.6%)

estimated precisely with coefficients of variation ranging between 3 and 36% (Table 3). Interindividual variability was identified for clearance (*CL*), the volume of the second compartment (*V2*) and the volume of the third compartment (*V3*) and correlations between the values of IIV were evaluated by using a full omega matrix. A significant correlation was obtained for *CL* and *V3* and this correlation was estimated in the final model. Validation of the pharmacokinetic model for S(-)-atenolol was performed by a posterior predictive check and a bootstrap analysis. The population estimates were nearly identical to the estimates obtained from the bootstrap replicates (Table 3). In addition the visual predictive check showed that the population PK model could well predict the time course of S(-)-atenolol in rats (Figure 3).

Pharmacodynamics The PD data were evaluated by means of population PKPD modelling. A sigmoidal Emax model was used to describe the concentration-effect relationship in both groups and an effect compartment was used to resolve the observed hysteresis in the effects of S(-)-atenolol on the heart rate.

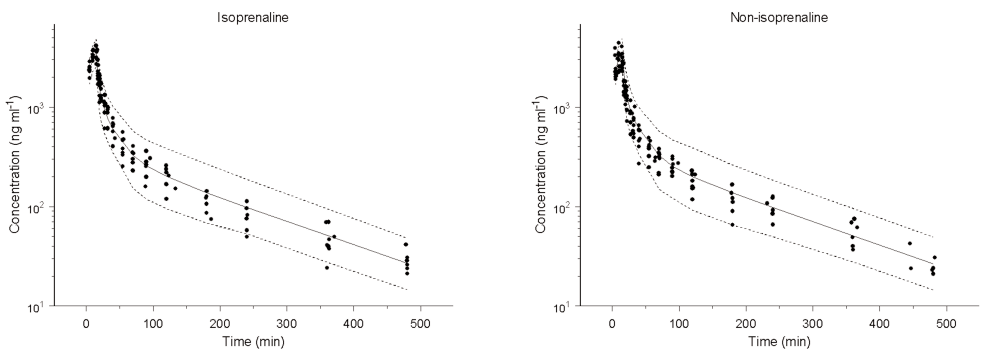

Figure 3, Visual predictive check population PK model for S(-)-atenolol. The range between the dashed lines depicts the 90% inter-quantile range. The solid line presents the population prediction. The solid dots are the observed concentrations.

Table 4, Population estimates of PD parameters and variabilities for S(-)-atenolol with (ISO) and without (non-ISO) isoprenaline-induced tachycardia.

Parameter	Value ISO (CV%)	Value non-ISO (CV%)
Structural parameters		
E_0 *(bpm)*	517 (2.6%)	362 (5.5%)
E_{max} *(bpm)*	-168 (8.8%)	-43.0 (41.2%)
EC_{50} *(ng ml^{-1})*	49.0 (28.8%)	27.9 (97.1%)
Ke0 (min^{-1})	0.042 (28.1%)	NE
n	0.950 (36.3%)	NE (1 FIX)
Interindividual variability (IIV)		
ω_{E0}^2	297 (50.1%)	1380 (55.4%)
ω_{Emax}^2	1860 (49.5%)	913 (55.9%)
Residual error		
σ_{PD}^2 *(bpm^2)*	747 (21.6%)	250 (23.2%)

In contrast to the PK data, the PD data were analysed separately, since the baseline heart rate was dissimilar between the isoprenaline and the non-isoprenaline group.

As expected the precise estimation of the population PD parameters in the non-isoprenaline group was more difficult than in the isoprenaline group (Table 4). Although it was possible to obtain estimates of E_0 (362.0 ± 20 bpm), E_{max} (43 ± 17.7 bpm) and EC_{50} (28 ± 27 ng ml^{-1}), the coefficient of variation for the EC_{50} was 97%. On top it was not possible to estimate a hill coefficient (*n*) and k_{eo} in the non-isoprenaline group, since incorporation of these parameters in the PD model resulted in increased imprecision in the EC_{50}. Inter individual variability was identified for E_0 and E_{max} for this group. The individual plots for the non-isoprenaline group are depicted in figure 4. Upon administration of S(-)-atenolol some individual rats show a clear decrease in heart rate (i.e. ID 8), while in others the drug effect was difficult to distinguish from normal variations in heart rate (i.e. ID 3).

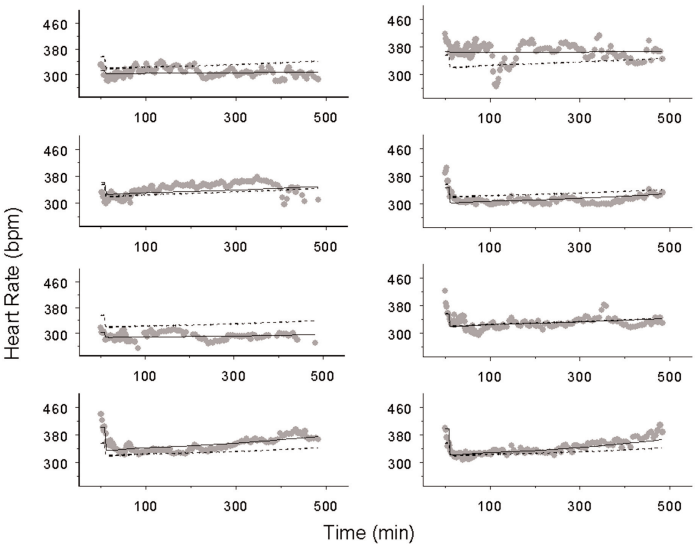

Figure 4, Individual plots pharmacodynamics S(-)-atenolol in the rat without isoprenaline-induced tachycardia. The solid line, the dashed line and the symbols represent the individual prediction, the population prediction and the heart rate observations respectively.

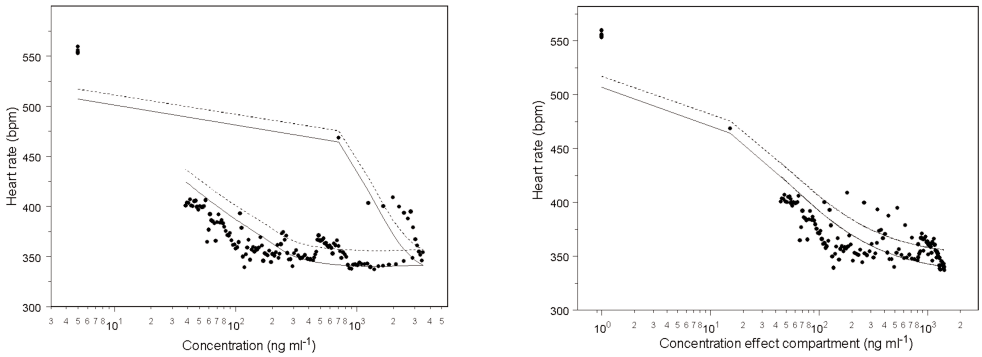

Figure 5, Concentration-effect relationship for S(-)-Atenolol for a typical individual rat with isoprenaline-induced tachycardia. A. Hysteresis loop – observed heart rate plotted against the concentration in plasma (Cp) B. Collapsed hysteresis loop – observed heart rate plotted against the concentration in the effect compartment (Ce). The solid line, the dashed line and the symbols represent the individual prediction, the population prediction and the heart rate observations respectively.

PKPD analysis of the isoprenaline group resulted in precise estimates for E_0 (517 ± 13 bpm), E_{max} (168 ± 15 bpm), EC_{50} (49 ± 14 ng ml^{-1}), n (0.95 ± 0.34) and k_{eo} (0.042 ± 0.012 min^{-1}) with acceptable coefficients of variation ranging from 4 to 50.1% (Table 4). Similar to the non-isoprenaline group IIV was observed for E_0 and E_{max}. With the use of an effect compartment model, the counter clockwise hysteresis loop collapsed and the equilibration half-time between the central and the effect compartment ($t_{1/2}$ = ln 2/k_{eo}) was 16.5 min (Figure 5). The individual fits of the model for the isoprenaline group are depicted in figure 6.

3.4 Discussion and conclusion

The overall aim of our research is to develop a mechanism-based PKPD model for β-blockers, with special reference to *in vitro-in vivo* correlations and the role of plasma protein binding. To this end the exact

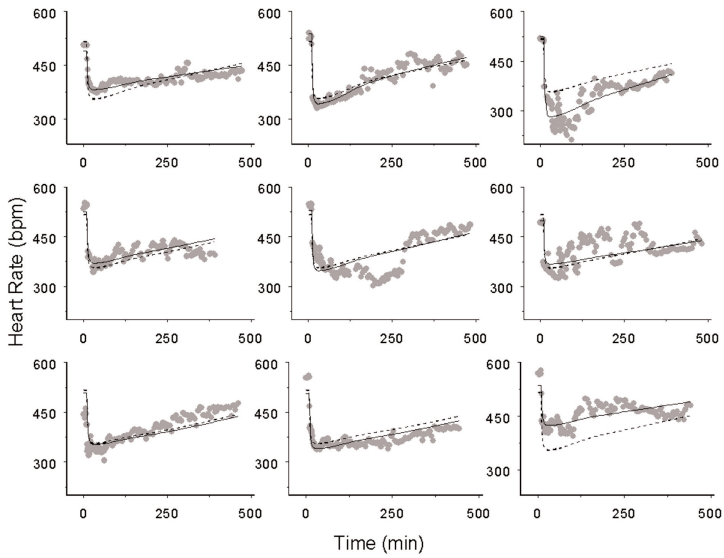

Figure 6, Individual plots pharmacodynamics S(-)-atenolol under Isoprenaline-induced tachycardia. The solid line, the dashed line and the symbols represent the individual prediction, the population prediction and the heart rate observations respectively.

quantification of the PD effects of β-blockers is important, and should be evaluated and validated carefully prior to the actual PKPD studies. In the present study the heart rate under continuous isoprenaline-induced tachycardia as a PD endpoint for β-blockers was validated. The results confirm that reduction of heart rate under isoprenaline-induced tachycardia provides a reliable and reproducible PD measurement for S(-)-atenolol in pre-clinical PKPD studies with a small number of individual rats. In addition it is shown that the measurement of the heart rate effects of S(-)-atenolol without isoprenaline-induced tachycardia results in imprecise estimation of the potency of the drug (EC_{50}).

The β-blockers are suitable compounds for investigation of the influence of drug specific properties on *in vivo* drug action. A lot is known about the β-blockers, including the interaction with the β-adrenoceptor and subsequent effect on heart rate (Reiter, 2004). In addition a wide array of different β-blockers is available which differ widely in physico-chemical properties and receptor affinity. Moreover, a continuous PD endpoint (heart rate) is readily available in both humans and laboratory animals. The effect on heart rate however is small and therefore difficult to describe accurately (Hoffman and Lefkowitz, 1996). Therefore optimisation and validation of the PD measurement for β-blockers is needed.

Isoprenaline- and exercise-induced tachycardia can both be used to improve the PD measurement for the β-blockers. Compared to exercise, induction of tachycardia with isoprenaline is easy achievable in experimental animals and the heart rate response is controllable. On top, the pharmacokinetics of isoprenaline in plasma are known (or can be measured) and this is an advantage for PKPD modelling.

To our knowledge, the PK and PD of isoprenaline in rats have not been reported in literature. Therefore the establishment of a PKPD model for this agonist was included in this study. The concentration-time profiles for isoprenaline were described using a two-compartment model and all parameters were estimated with good precision. Isoprenaline-induced tachycardia in the PKPD studies for S(-)-atenolol is obtained with a 5 μg kg^{-1} h^{-1} intravenous infusion. The steady state concentration of isoprenaline for a typical rat of 294 g is 0.23 ng ml^{-1}. The induction of tachycardia at a dose of 5 μg kg^{-1} h^{-1} thus approximates the maximal effect of isoprenaline (figure 2).

The tachycardia produced by isoprenaline is primarily due to β-adrenoceptor activity in the heart (Chiu *et al*., 2000). Although β_1, β_2 and β_3 adrenoceptor are present in mammalian heart, the positive chronotropic effect of isoprenaline *in vivo* are brought about by β_1-adrenoceptors (Nandakumar *et al*., 2005; Piercy, 1988; Wellstein *et al*., 1987). It is occasionally suggested that β_2-adrenoceptors are also involved in the effect on heart rate, however only a small population of functional β_2-adrenoceptors is present in the heart of WKY rats (Doggrell and Surman, 1994). Although no *in vivo* potencies in rat have been reported, isoprenaline is considered an extremely potent β-adrenoceptor agonist and this is confirmed in the present study with an *in vivo* potency (EC_{50}) of 0.014 ng ml^{-1} (Waldeck, 2002).

For the development of PKPD models a continuous measure of drug effect is preferable (i.e. heart rate, EEG, body temperature). For that reason we investigate the use of a continuous intravenous infusion of isoprenaline in the PD measurement of S(-)-atenolol (Dingemanse *et al*., 1988). With the use of continuous isoprenaline-induced tachycardia the effect on heart rate of S(-)-atenolol is clearly discernible form normal variations in heart rate and non-stop recording of the pharmacological effect is achievable.

The concentration-time profiles of S(-)-atenolol with and without isoprenaline-induced tachycardia were analysed simultaneously since no differences were found in the PK between both groups (data not shown). The PK parameters obtained from the description of the profiles by a three compartment model are comparable with literature (Mehvar *et al*., 1990; Belpaire *et al*., 1990; Belpaire *et al*., 1993; de Lange *et al*., 1994). In literature however, the plasma concentrations of atenolol following intravenous administration are described by a two-compartment model. This difference might be explained by the

duration of the experiments, which is usually 2-3 h in literature compared with 8 h in our experiment.
In this study, we compared the PD of S(-)-atenolol with and without isoprenaline-induced tachycardia. Some individual profiles in both the isoprenaline and non-isoprenaline group display a large variation in heart rate (i.e. ID: 2; ID:11 and ID:14) which is caused by the measurement. Since the heart rate is captured from a cannula in the femoral artery, blood cloths in the cannula sometimes disturb the signal.
The reduction of isoprenaline-induced tachycardia provides a robust PD endpoint for the β-blockers in preclinical investigations. The concentration-effect relationship is described with a sigmoid Emax model and contrary to the non-isoprenaline group all PD parameters including inter individual variability on E_0 and E_{max} are estimated precisely.
Baseline heart rate in the non-isoprenaline group is 362 ± 20 bpm and the maximal effect is a reduction of 43 ± 18 bpm. The variation in heart rate due to circadian rhythms, movement and stress are approximately 30 bpm in rats (Oliveira *et al*., 2004; Lacchini *et al*., 2001). The maximal effect of S(-)-atenolol on resting heart rate is, thus, only slightly larger than the observed variability in heart rate. For that reason exact quantification of the concentration-effect relationship is complicated in a small number of individuals and thus results in uncertainty in the estimation of potency (EC_{50}). Furthermore, the interanimal variability in baseline heart rate approximates the maximal drug effect. In the isoprenaline group, the baseline heart rate is 517 ± 13 bpm and the maximal reduction in heart rate is 168 ± 15 bpm. The maximal effect with isoprenaline-induced tachycardia is thus clearly distinguishable from the variability in heart rate.
As expected the EC_{50} of S(-)-atenolol in the isoprenaline group (49 ± 14 ng ml^{-1}) is larger than in the non-isoprenaline group (28 ± 27 ng ml^{-1}) due to the presence of a higher concentration of the agonist in the system (Rang *et al*., 1999).
A remarkable finding was the hysteresis observed in the concentration-effect relationship and thus the need for an effect compartment in the PKPD model (figure 5). Although the use of an effect compartment for β-blockers is not uncommon in literature for the effect on blood pressure, the effect on heart rate is assumed to be an acute effect since the β_1 receptor is present in the plasma compartment (Brynne *et al*., 2000; Hocht *et al*., 2004; Hocht *et al*., 2006; Ritchie *et al*., 1998). The equilibration half-time between the central compartment and the effect compartment ($t_{1/2}$) in this study is 16.5 min. Hocht et al., studied the effect of metoprolol on heart rate in WKY rats and reported an equilibration half-time of 36.6 and 20.4 min for 3 and 10 mg kg^{-1} metoprolol respectively (Hocht *et al*., 2006). The delay in effect on heart rate for metoprolol is slightly larger than for S(-)-atenolol, which might be the result of the difference in lipophilicity between both drugs. In theory, hysteresis in the effect can be a consequence of biophase equilibration, receptor association and transduction processes. It has been suggested in literature that the myocardial time-concentration profile more closely resembles time-response profile for the acute pharmacodynamics effect of cardioactive drugs (Ritchie *et al*., 1998; Anderson *et al*., 1980). Biophase equilibration may ,therefore, be one of the explanations for the observed hysteresis in this study. On the other hand, the observed hysteresis might be the result of the interaction of the β-blocker with the agonist at the receptor. It is known that in order to obtain a maximal increase in heart rate by isoprenaline, a receptor occupancy of only 5% is needed. Thus although atenolol displaces isoprenaline from the receptor, the interaction does not decrease heart rate immediately.
A reproducible and reliable method for the PD measurement of S(-)-atenolol *in vivo* in rats has been developed. This method allows the continuous measurement of the effect of atenolol and other β-blockers on heart rate under isoprenaline-induced tachycardia and can be used in the development of mechanism-based PKPD models.

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