Towards predictive cardiovascular safety : a systems pharmacology approach

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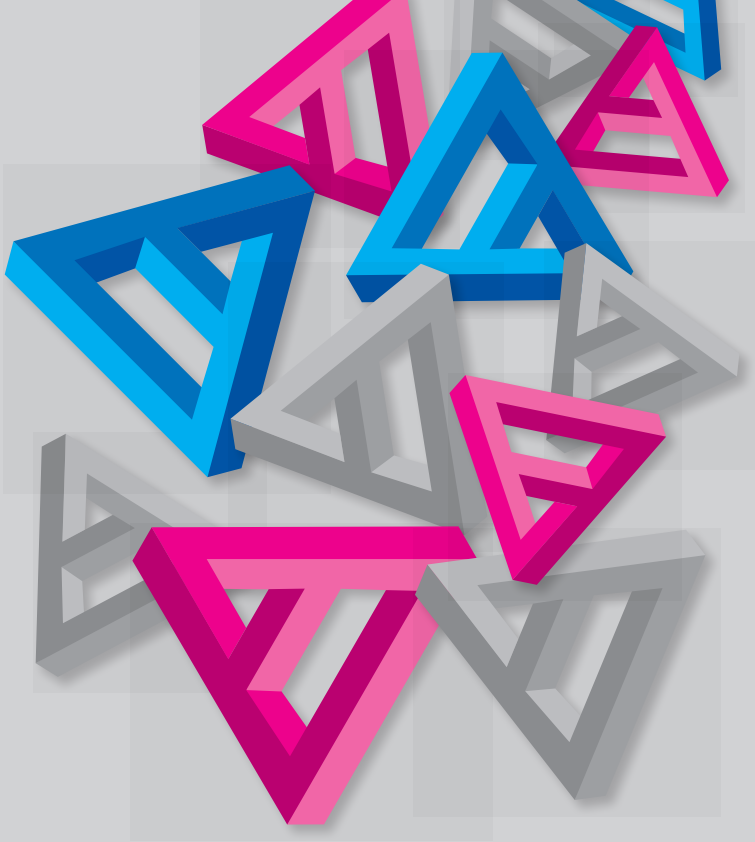

CHAPTER 3

PKPD modeling of the interrelationship between mean arterial blood pressure, cardiac output and total peripheral resistance in conscious rats

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Summary

Background and purpose | The homeostatic control of arterial blood pressure is well understood with changes in blood pressure (BP) resulting from changes in cardiac output (CO) and/or total peripheral resistance (TPR). Drug effects on this interrelationship have not been analyzed in a mechanism-based and quantitative manner. This is important since it may constitute a basis for the prediction of drug effects on BP. This investigation aimed to describe, in a mechanism-based and quantitative manner, the effects of drugs with different mechanisms of action (MoA) on the interrelationship between BP, CO and TPR.

Experimental approach | The cardiovascular effects of 6 drugs with diverse MoA's, (amlodipine, fasudil, enalapril, propranolol, hydrochlorothiazide and prazosin) were characterized in spontaneously hypertensive rats. The rats were chronically instrumented with ascending aortic flow probes and/or aortic catheters/radiotransmitters for continuous recording of CO and/or BP. Data were analyzed in conjunction with independent information on the time course of drug concentration using a mechanism-based PKPD modeling approach.

Key results | By simultaneous analysis of the effects of 6 different compounds, the dynamics of the interrelationship between BP, CO and TPR, were quantified. System-specific parameters could be distinguished from drug-specific parameters indicating that the developed model is drug-independent.

Conclusions and Implications | A system-specific model characterizing the interrelationship between BP, CO and TPR has been obtained, which can be used to quantify and predict cardiovascular drug effects and to elucidate the MoA for novel compounds. Ultimately, the proposed PKPD model may allow prediction of BP effects in humans based on preclinical data.

Introduction

Persistent elevation of blood pressure (BP) is a risk factor for heart failure and is a leading cause of cardiovascular disease (Graham *et al.*, 2007). This risk continuously increases with the level of BP. Even small changes in BP, i.e. 10-20 mmHg, can have a relatively large influence (EMEA, 2004). BP regulation by the cardiovascular system (CVS) is well characterized, and the homeostatic principles of the CVS are thoroughly understood. Briefly, mean arterial pressure (MAP) equals the product of cardiac output (CO) and total peripheral resistance (TPR). This relationship has been well established for many years and is based on Ohm's Law, when applied to fluid flow. MAP is maintained within narrow limits by various regulatory feedback systems such as the renin-angiotensin-aldosterone system (RAAS) and the baroreflex system (Cleophas, 1998). In contrast to the detailed understanding of the physiologic regulation of BP, the mechanisms underlying the desired or undesired drug effects on BP are often less clear. This is a major drawback since a quantitative understanding of the pharmacological effects of (novel) drugs on BP control is pivotal with regard to drug efficacy and safety. Moreover, understanding these effects early in preclinical development could improve the anticipation of the magnitude of hemodynamic effects in humans.

To date no models exist that provide an integrated description of the effects of drugs on the interrelationship between MAP, CO and TPR. A mechanism-based pharmacokinetic-pharmacodynamic (PKPD) modeling approach is uniquely suited to provide quantitative insights in drug effects on the CVS since it clearly distinguishes drug-specific properties from system-specific properties (Danhof *et al.*, 2007; Ploeger *et al.*, 2009). This separation enables prediction and extrapolation of treatment effects to later stages of development using a translational modeling approach and, thereby, facilitating the drug development process and supporting compound selection (Danhof *et al.*, 2007).

Following the concepts proposed by Van Der Graaf *et al.* (1999) and Van Schaick *et al.* (1997), we hypothesize that by challenging the CVS with a variety of compounds the rate and feedback parameters of the CVS can be quantified and a clear distinction can be made between drug- and system- specific parameters that govern the pharmacological effect. A crucial factor is that the 'training set' of selected compounds acts on the same system, but with different target sites and time courses of effect. We have selected a training set of six antihypertensive compounds with different, but well described, effects on CO and/or TPR: enalapril, fasudil, amlodipine, prazosin, propranolol and hydrochlorothiazide (HCTZ) to challenge the CVS. The first four compounds have their primary effect on TPR; whereas the last two compounds have their primary effect on CO (Cleophas, 1998; Masumoto *et al.*, 2001; Ram *et al.*, 1981). An overview of the MoA of these compounds can be found

in Table 1. Besides an adequate selection of compounds, another important aspect of the experimental design is the selection of endpoints to monitor the drug effects on the CVS. Measuring BP is common practice, but it represents a 'secondary' pharmacodynamic parameter, as BP depends on both CO and TPR. At present, measuring CO has not been integrated into daily practice due to difficulties associated with invasive instrumentation procedures (Doursout *et al.*, 2001). Still, from a mechanistic point of view these data are pivotal for a quantitative understanding of the dynamics of the system, especially since, due to the homeostatic feedback mechanisms, the effects on the underlying parameters CO and TPR may be much larger than the effects on BP (Brands *et al.*, 2000). Finally, monitoring BP during the onset and offset of the drug effects provides the information

Table 1: Selected compounds to challenge the CVS with the aim of distinguishing system- from drug-specific parameters and their mechanism of action.

Compound	Class	Mechanism of action	Primary effect
enalapril	angiotensin-converting enzyme (ACE) inhibitor	ACE inhibitors competitively inhibit angiotensin I-converting enzyme, preventing the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor that also stimulates release of aldosterone. Decreased levels of angiotensin II lead to decreased total peripheral resistance that is unassociated with reflex stimulation of the heart (Frohlich, 1989).	TPR
fasudil	rho-kinase inhibitor	Rho-kinase inhibits myosin light chain phosphatase activity and plays a key role in Ca^{2+} sensitization and hypercontraction of vascular smooth muscle cells. Rho-kinase inhibitors decrease total peripheral resistance (Masumoto *et al.*, 2001).	TPR
amlodipine	calcium channel blocker	Amlodipine is a dihydropyridine that blocks voltage gated calcium channels and selectively inhibits Ca^{2+} influx into vascular smooth muscle cells. Calcium antagonists act by decreasing total peripheral resistance to lower arterial pressure. As a consequence, reflex tachycardia, increased cardiac output, and increased plasma catecholamine and plasma renin activity are commonly seen, particularly with the initial dose and with short-acting dihydropyridines (Michalewicz and & Messerli, 1998; Perez-Reyes *et al.*, 2009)	TPR
prazosin	selective α_1 adrenergic receptor blocker	Prazosin is a quinazoline derivative that is a specific and selective competitive antagonist of α_1 adrenoceptors on vascular smooth muscle cells. Prazosin reduces BP by reducing elevated peripheral resistance and has little effect on cardiac function (Reid *et al.*, 1987).	TPR
propranolol	β-adrenergic receptor blocker	Propranolol is a non-selective beta blocker. It antagonizes the action of norepinephrine and epinephrine at all β-adrenergic receptors. Propranolol decreases cardiac output and heart rate with a reflex rise in total peripheral resistance (Ebadi, 2008).	CO
HCTZ	diuretic	Diuretics cause blood volume contraction and lower venous pressure, which decreases cardiac filling and, by the Frank-Starling mechanism, decreases ventricular stroke volume (Levick, 2003).	CO

needed to quantify the parameters of a dynamical system as this information can only be derived when the system is not in equilibrium. The offset phase can be especially informative as it provides information on the question if, and how fast, the system returns to its initial state.

In this investigation, we describe the development of a mechanism-based PKPD model that integrates a quantitative description of the physiology of the interrelationship between BP, CO and TPR and the pharmacological effects of cardiovascular drugs using data from preclinical experiments with a training set of six antihypertensive drugs. Ultimately, this quantitative pharmacology model may be used to predict clinical responses to novel pharmacologic agents.

Methods

Animals

Experiments were conducted on male, spontaneously hypertensive rats (SHR) (Taconic Farms,Germantown, NY) in accordance with approved Novartis Animal Care and Use Committee protocols and the Guide for the Care and Use of Laboratory Animals. At the time of study, rats' ages ranged from 21-45 wk and body weights ranged from 269-490 gram. Rats were housed on a 12-h light/dark cycle (light: 6 am to 6 pm) and were provided normal chow (Harlan Teklad 8604; Indianapolis, IN) and water ad libitum. The total number of rats used was 12 (10 in Study 1 and 2 in Study 2). All studies involving animals are reported in accordance with the ARRIVE guidelines for the reporting of experiments involving animals (Kilkenny et al., 2010; McGrath et al., 2010).

Experimental Procedures

The effects of a training set of compounds were obtained in two studies. In Study 1, detailed profiles of the time-course of the effects on MAP and HR were obtained after repeated dosing. In Study 2, information on the effect on MAP and CO was obtained following a single administration of a range of different doses. The combined information from both studies was crucial to the identification of the system-specific model characterizing the interrelationship between MAP, CO and TPR.

For the recording of BP (Study 1), a sterile gel-filled catheter/radiotransmitter (PA-C40, Data Sciences International, St. Paul, MN) was surgically implanted under isoflurane anesthesia into a femoral artery (catheter tip residing in the lower abdominal aorta) and a

subcutaneous pocket or directly into the abdominal aorta. Arterial BP was for recorded for 15 sec every 10 min as detailed previously (Bazil *et al.*, 1993).

For BP and CO measurement (Study 2), rats were surgically instrumented with both an ascending aortic flow probe and a femoral arterial catheter/radiotransmitter (Figure 1). Rats were anesthetized with isoflurane, tracheally intubated, and artificially ventilated. A pre-calibrated 2.5 mm or 3.0 mm transit-time volumetric flow probe (2.5PS or 3PS, Transonic Systems Inc., Ithaca, NY) was placed around the ascending aorta via an incision at the right second intercostal space. The flow probe connector was tunneled subcutaneously to the mid-scapular region, where it was attached to the skin by a cutaneous button. The ribs were approximated with sutures, the chest was evacuated of air, and the chest wound closed in layers. Ketoprofen and penicillin G were administered for analgesia and infection prophylaxis. The rat was extubated and allowed to recover for approximately two weeks. Thereafter, the catheter/radiotransmitter was implanted as described above.

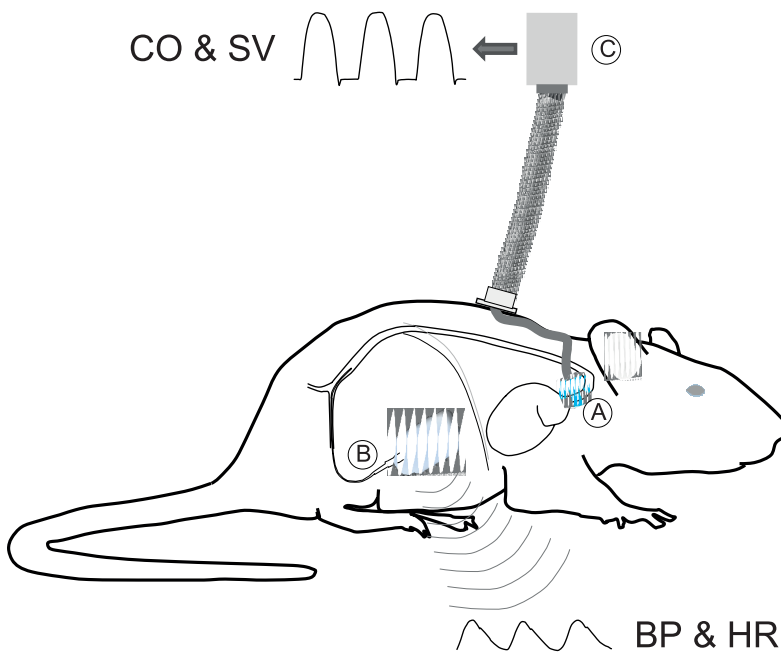

Figure 1: Experimental animal instrumentation. Rats in Study 2 were surgically instrumented with both an ascending aortic flow probe (A) and a femoral arterial catheter/radiotransmitter (B). CO was measured by connecting the flow probe to the flow meter via a cable and electrical swivel (C), which allowed the animal to remain fully ambulatory. MAP, heart rate, stroke volume, CO, and TPR were derived for all beats averaged over consecutive 2-min intervals.

Table 2: Study overview

Study	Measures	Study designs	Compound	Dose	Number of rats
1 Multiple dosing study	MAP	Days -1 - 0 : baseline (not included in analysis) Day 1 : baseline Days 2 - 3 : vehicle Days 4 - 9 : active treatment (once daily) Days 10 - 15: washout	enalapril	30 mg/kg p.o	5
			fasudil	30 mg/kg p.o	5
			amlodipine	10 mg/kg p.o	5
			propranolol	1 mg/mL in drinking water	5
2 Single administrations of different doses on separate days	MAP, CO (and TPR)	Day 1: vehicle Days 2-5: a different dose each day	amlodipine	0.3, 1, 3, 10 mg/kg p.o.	2
			prazosin	0.04, 0.2, 1, and 5 mg/kg p.o.	2
			HCTZ	0.1, 0.3, 1, 3 mg/kg p.o.	2

In Study 2, rats were used repeatedly for up to 6 months with sufficient washout between consecutive experiments. For continuously recording of cardiac output, a tether cable was attached to the flow probe connector and a flow meter (Model T402, Transonic Systems) via an electrical swivel (Dragonfly Research & Development, Ridgeley, WV). The digitized flow and telemetered pressure signals were analyzed by a Ponemah data acquisition system (Data Sciences International). MAP, heart rate, stroke volume, CO, and TPR were derived for all beats averaged over consecutive 2-min intervals.

Experimental design

Two different studies were conducted (Table 2). In Study 1, rats were treated once daily for 6 days with a single dose of drug (enalapril, fasudil, amlodipine or propranolol); SHR, n=5/drug. In Study 2, rats received single administrations of 4 different doses of each drug (amlodipine, prazosin or HCTZ) on 4 separate days.

In Study 1, rats were telemetered and after 2 weeks recovery, received 1 week of daily, oral dosing of saline (dosing training), then baseline data were collected during 3 days of no treatment. Subsequently, rats were treated with vehicle for 2 days prior to active treatment with active drug, which was administered once daily for 6 days at 11.00 am. Thereafter, washout data were collected during 6 days.

In Study 2, flow cables were connected to the flow probes by 7:00 am and disconnected after 5:00 pm. Baseline data were collected between 8:00 am and 10:00 am each day. Rats were dosed at 10:00 am and all data were continued to be collected until 5:00 pm. Thereafter, only MAP and HR data were captured until the flow probes were reconnected the next morning.

Compounds

In Study 1, enalapril maleate (Sigma-Aldrich, St. Louis, MO, USA, E6888), fasudil mono HCl (LC Laboratories, Woburn, MAF-4660), and amlodipine besylate (Lek pharmaceuticals d.d., Verovskova, Ljubljana, Slovenia) were formulated for administration at 5 ml/kg by oral gavage. (±)-Propranolol HCl (Sigma-Aldrich, P0884) was dissolved in drinking water at 1 mg/mL. Enalapril maleate, fasudil and amlodipine were homogenized in 0.5% methylcellulose (MC) (Fisher Scientific, Pittsburgh, PA).

In Study 2, prazosin HCl (Sigma-Aldrich, P7791), amlodipine besylate, and HCTZ (H2910, Sigma-Aldrich) were formulated for administration at 2 ml/kg by oral gavage. Prazosin and amlodipine were homogenized in 0.5% MC whereas HCTZ was dissolved in NaOH and diluted with filtered water (vehicle was water adjusted to pH 11).

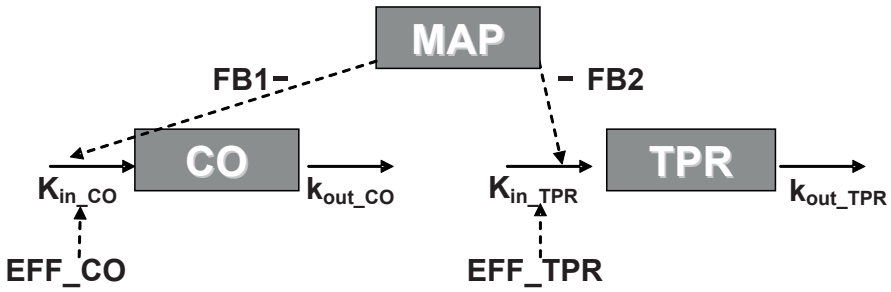

Figure 2: Cardiovascular model to describe the change in mean arterial BP after administration of different compounds acting on cardiac output (CO) and total peripheral resistance (TPR). MAP equals the product of CO and TPR (MAP=CO*TPR). Effects on CO and TPR are described by two linked turnover equations. When MAP increases as a result of a stimulating effect on CO or TPR, the values of CO and TPR will decrease as a result of the action of the different feedback mechanisms regulating the CVS. *The magnitude of feedback on CO and TPR is represented by FB1 and FB2.* K_{in_CO} *and* K_{in_TPR} *represent the zero-order production rate constants of CO and TPR and* k_{out_CO} *and* k_{out_TPR} *represent the first-order dissipation rate constants of CO and TPR.*

Data analysis

The interrelationship between MAP, CO and TPR is expressed in the formula: MAP=CO*TPR (Levick, 2003). On the basis of this relationship a model was developed to describe the time course of the effects on MAP, CO and TPR (Figure 2). The model was defined by two linked turnover equations involving CO and TPR (Equation 1). Turnover models are also called indirect response models and can be used to describe hysteresis, i.e. the delay between a perturbation and a response (Dayneka *et al.*, 1993). Examples of applications of this type of model can be found in the modeling of the homeostatic features of the release of endogenous compounds such as hormones or proteins (Gabrielsson and Weiner, 2000), or in the modeling of pharmacological responses such as drug-induced hypothermia (Zuideveld *et al.*, 2001).

$$\frac{dCO}{dt} = K_{in\_CO} \cdot (1 - FB1 \cdot MAP) - k_{out\_CO} \cdot CO$$
$$\frac{dTPR}{dt} = K_{in\_TPR} \cdot (1 - FB2 \cdot MAP) - k_{out\_TPR} \cdot TPR \quad (1)$$

$$MAP = CO \cdot TPR$$

In these equations, K_{in_CO} and K_{in_TPR} represent the zero-order production rate constants and k_{out_CO} and k_{out_TPR} represent the first-order dissipation rate constants of CO and TPR respectively. These hypothetical production and dissipation rate constants reflect the rate of change in CO and TPR. *FB1* and *FB2* are constants representing the magnitude of the

negative feedback of MAP on CO and TPR. Following the criteria for statistical significance as specified in the section "Computation", linear relationships between MAP and the production rate constants of CO and TPR were the most parsimonious relationships that captured the feedback mechanism adequately.

Initially, the circadian rhythm in BP was described as the sum of a maximum of 10 harmonics with different periods (Equation 2). The number of cosine functions was systematically reduced following the criteria for statistical significance (section "Computation").

$$MAP = CO \cdot TPR + \sum_{n=1}^{10} amp_n \cdot \cos\left(\frac{n \cdot 2\pi \cdot (t + hor)}{24}\right) \quad (2)$$

In this equation, *amp* represents the amplitude, *t* the time and *hor* the horizontal displacement over time. From a mechanistic point of view it is expected that the circadian rhythm in BP is a result of a circadian rhythm in CO and/or TPR as these are the primary drivers of MAP. However, as no 24 h measurements could be obtained for CO and TPR, the circadian rhythm was included in the model on MAP. Before pharmacological intervention (at baseline), MAP oscillates around its baseline value, which equals the product of the baseline values of CO and TPR *(BSL_CO* and *BSL_TPR).*

Before pharmacological intervention, the system is in steady state, or dynamic equilibrium in mathematical terminology, denoting that MAP, CO and TPR do not change over time and are equal to their baseline values. As is common practice for turnover models (Dayneka *et al.*, 1993) steady state conditions are described by the following equations (Equation 3) in which K_{in} is expressed in terms of *BSL* and k_{out}.

$$K_{in\_CO} = \frac{-k_{out\_CO} \cdot BSL\_CO}{-1 + FB1 \cdot BSL\_CO \cdot BSL\_TPR}$$

$$K_{in\_TPR} = \frac{k_{out\_TPR} \cdot (K_{in\_CO} \cdot FB1 \cdot BSL\_TPR + k_{out\_CO}) \cdot BSL\_TPR}{K_{in\_CO} \cdot FB1 \cdot BSL\_TPR + k_{out\_CO} - FB2 \cdot K_{in\_CO} \cdot BSL\_TPR} \quad (3)$$

In the experiments, TPR was derived (Equation 1) from the directly measured MAP and CO. In contrast, in the modeling, the baseline values of MAP (*BSL_MAP*) and *BSL_TPR* were estimated and *BSL_CO* was derived from these parameters for reasons of model stability. The system was functionally characterized by challenging the CVS with six different drugs with different mechanisms of action. Drug effects (*EFF*) were assumed to influence the production rates of either CO or TPR according to Equation 4.

$$\frac{dCO}{dt} = K_{in\_CO} \cdot (1 - FB1 \cdot MAP - EFF) - k_{out\_CO} \cdot CO$$
$$\frac{dTPR}{dt} = K_{in\_TPR} \cdot (1 - FB2 \cdot MAP - EFF) - k_{out\_TPR} \cdot TPR \quad (4)$$

During pharmacological intervention TPR and CO can be calculated using Equation 5, where TPR_{ss} and CO_{ss} represent TPR and CO at steady state.

$$a = k_{out\_TPR} \cdot K_{in\_CO} \cdot FB1$$
$$b = K_{in\_TPR} \cdot K_{in\_CO} \cdot FB2 + k_{out\_TPR} \cdot k_{out\_CO} + K_{in\_TPR} \cdot K_{in\_CO} \cdot FB1 \cdot (EFF - 1)$$
$$c = K_{in\_TPR} \cdot k_{out\_CO} \cdot (EFF - 1) \quad (5)$$
$$TPR_{ss} = \frac{(-b + \sqrt{b^2 - 4 \cdot a \cdot c})}{2 \cdot a}$$
$$CO_{ss} = \frac{K_{in\_CO}}{K_{in\_CO} \cdot FB1 \cdot TPR_{ss} + k_{out\_CO}}$$

Linear, E_{max} and Sigmoid E_{max} models were evaluated to describe the drug effects on CO or TPR. The effects of all compounds were best described by E_{max} models (Equation 6):

$$EFF = \frac{E_{max} \cdot C(t)}{EC_{50} + C(t)} \quad (6)$$

In this equation, E_{max} and EC_{50} represent the maximum effect and the concentration resulting in a half-maximal effect, respectively, and C equals the drug concentration in plasma, which varies with time. Using the time course of the drug plasma concentrations, i.e., the pharmacokinetics (PK), rather than the dose or exposure, as a predictor for the pharmacodynamics (PD) has the advantage that it enables a better description of the time course of the drug effect. As the PK was not measured in these experiments, predicted plasma concentration *versus* time profiles were derived from the literature (Table 3). However, experimental conditions and formulations were different in these literature studies as compared to the experiments described in this paper. Therefore, for some compounds, PK parameters, e.g. the absorption rate, were estimated based on the known other PK parameters and the effect on BP (Table 3). In that case, PK and PD parameters were estimated simultaneously.

Table 3: Specification of the PK models to describe the pharmacokinetics of the six selected compounds, enalapril, fasudil, amlodipine, prazosin, propranolol and HCTZ to challenge the CVS. The PK models were based on literature models. The adjustments required to account for the differences in experimental conditions and formulations in these literature studies as compared to the experiments described in this paper are described in the "Comments" column.

Compound	PK model	Literature model	Comments	Species
enalapril	2-compartmental model with Michaelis-Menten elimination	(Lin *et al.*, 1988)	Data read out from the manuscript and a 2- compartmental model with Michaelis-Menten elimination was optimized in NONMEM	Sprague-Dawley rats
fasudil	1-compartmental model	(Ikegaki *et al.*, 2001): Non-compartmental analysis	*Ka* and lag-time were derived from the reported half-life, AUC and C_{max} using Berkeley Madonna	Wistar-Kyoto rats
amlodipine	1-compartmental model	(Stopher *et al.*, 1988): Non-compartmental analysis	Ka was derived from the reported half-life, *Vd*, *F* and T_{max} using Berkeley Madonna	Sprague-Dawley rats
prazosin	1-compartmental model	(Hamilton *et al.*, 1985): 1-compartmental model	*CL*, *Vd*; scaled to rat using allometric scaling. Ka was estimated	New Zealand white rabbits
propranolol	3-compartmental model	(van Steeg *et al.*, 2010) 3-compartmental model	Absorption described as an infusion with a fixed duration of 24 h. *Ka* was estimated	Wistar-Kyoto rats
HCTZ	1-compartmental model	(Asdaq and Inamdar, 2009): 1-compartmental model	Reported: *Ke*, *Ka*, *Vd*, AUC -> *F* was calculated from these parameters	Wistar-Kyoto rats

CL: clearance
Vd: distribution volume
Ke: elimination rate
Ka: absorption rate
F: bioavailability

Assumptions

The PK and PD models were based on the assumptions described in Table 4.

Table 4: Model assumptions

No.	Assumption	Clarification
1	Compounds selectively influence either CO or TPR.	Although some compounds may have a combined mechanism of action, i.e., have an effect on both CO and TPR, it was assumed that only including the direct/primary effect was sufficient for identifying the system. Therefore, any changes observed in the other parameters were assumed to be a result of the feedback (indirect/secondary effect).
2	All compounds influence the production rates of CO or TPR rather than the dissipation rates.	This assumption is based on the MoA of the selected compounds (Table 1).
3	For compounds for which the maximum effect was not observed, complete inhibition (i.e., E_{max} = 1) was assumed at infinite concentrations to ensure identification of the EC_{50} parameter.	To evaluate the validity of this assumption, the influence of different values of the E_{max} (i.e. E_{max} =0.8) on the estimates of the system parameters was tested. This was done for one of the compounds (amlodipine).
4	The PK do not differ between rat strains and can be scaled between rabbit and rat on the basis of an allometric function (West *et al.* 1999; Anderson and Holford, 2009).	Although published information on the PK of all selected compounds was available, the PK was often evaluated in different rat strains and, for prazosin, even in a different species (rabbit).

Influence of the selection of compounds on the system-parameters

An adequate selection of compounds to challenge the functioning of the CVS was thought to be pivotal to successfully quantify the parameters of the CVS model. The compounds were selected to have different mechanisms and durations of action as this provides the maximum power to identify the model i.e. to distinguish system- and drug-specific parameters. Furthermore, we expected that a combined analysis of data from the six compounds would enable accurate and precise quantification of all system-parameters. To determine whether the obtained model is truly system specific, the influence of selectively omitting the data of one of the six compounds on the values of the system parameters was examined. If omission of these data does not lead to significant changes in these parameter estimates, this indicates that the model is truly drug-independent. In this analysis, the estimates of the system parameters obtained with these six sub-models were compared with those of the model based on all compounds.

System properties

Simulations were performed to investigate if the profiles of the time-course of the drug effect on MAP, CO and TPR are different for compounds with an influence on either CO or TPR. The typical profiles of MAP, CO and TPR *versus* time and of CO *versus* TPR are referred to as signature profiles. Pertinent differences in signature profiles for compounds with either an effect on CO or TPR indicate whether the drug-independent model can be applied to investigate the site of action (CO or TPR) of new compounds with an unknown MoA on BP. The responses on CO, TPR and MAP were simulated after triggering the model by enhancing TPR or inhibiting CO. The stimulation and inhibition functions were analyzed for a hypothetical constant rate infusion during 100 h to ensure that the drug effect is in steady state.

Computation

The data from Studies 1 and 2 were simultaneously analyzed using the non-linear mixed-effects modeling approach implemented in NONMEM (version 7.1.0; Icon Development Solutions, Ellicott City, Maryland, USA). The models were compiled using Digital Fortran (version 6.6C3, Compaq Computer Corporation, Houston, Texas) and executed on a PC equipped with an AMD Athlon 64 processor 3200+ under Windows XP. The results from the NONMEM analysis were subsequently analyzed using the statistical software package S-Plus for Windows (version 6.2 Professional, Insightful Corp., Seattle, USA). The simulations were carried out using Berkeley Madonna (version 8.3.5, Berkeley Madonna Inc., University of California). Parameters were estimated using the first order conditional estimation method with interaction between the two levels of stochastic effects (FOCE interaction). Random effects were included as exponential terms reflecting lognormal distributions of model parameters. The residual variability was explored with proportional and additive error models. Goodness-of-fit was determined using the minimum value of the objective function defined as minus twice the log-likelihood. For nested models, a decrease of 10.8 points in the objective function (MVOF) (corresponding to $p<0.001$ in a chi-squared distribution) by adding an additional parameter was considered significant. The goodness-of fit was also investigated by visual inspection of the plots of individual predictions and the diagnostic plots of (weighted) residuals. In addition, a visual predictive check was performed in which the median and the 90% inter-quantile range of data simulated with the developed model were plotted together with the observations.

Results

Model development

The CVS model as expressed by Equations 1 - 6 and graphically represented in Figure 2 was used to simultaneously analyze the data from Studies 1 and 2. To characterize the circadian variation in the baseline, the amplitudes of 5 harmonics of the circadian rhythm could be quantified. Amp_1, amp_3, amp_4, amp_5 and amp_7 were estimated to be 3.17, -2.03, 1.15, 1.63 and 1.28 mmHg, respectively. Amp_2, amp_6, amp_8, amp_9 and amp_{10} were fixed to 0 implying that these harmonics did not contribute to the circadian rhythm. In Study 1, *BSL_MAP* was allowed to vary between individual rats (inter-individual variability (IIV)). Study 2 provided information to estimate IIV on both *BSL_MAP* and *BSL_TPR*. The residual errors of MAP and TPR were best described by additive residual error models, whereas the residual error of CO was best described by a proportional error model. The dissipation rate of CO (k_{out_CO}) was found to be very high and could not be estimated with good precision. Therefore, this parameter was fixed to a high value (99 1/h) prior to estimating the other model parameters. The effects of all compounds were best described by E_{max} models. However, for amlodipine, fasudil, enalapril and HCTZ it was not possible to identify both drug effect parameters, E_{max} and EC_{50}, independently and with good precision. This was due to the fact that the maximum effect was not observed. Therefore, E_{max} was fixed to 1 for these compounds assuming that complete inhibition of K_{in} can be reached for infinite concentrations. For these compounds the drug effects could have also been described with a linear concentration-effect relationship. However, these models were not applicable as the inhibition of K_{in} exceeded 100% during parameter optimization. In addition, adding a sigmoidicity parameter to the E_{max} models did not result in an improvement in the goodness of fit for all compounds.

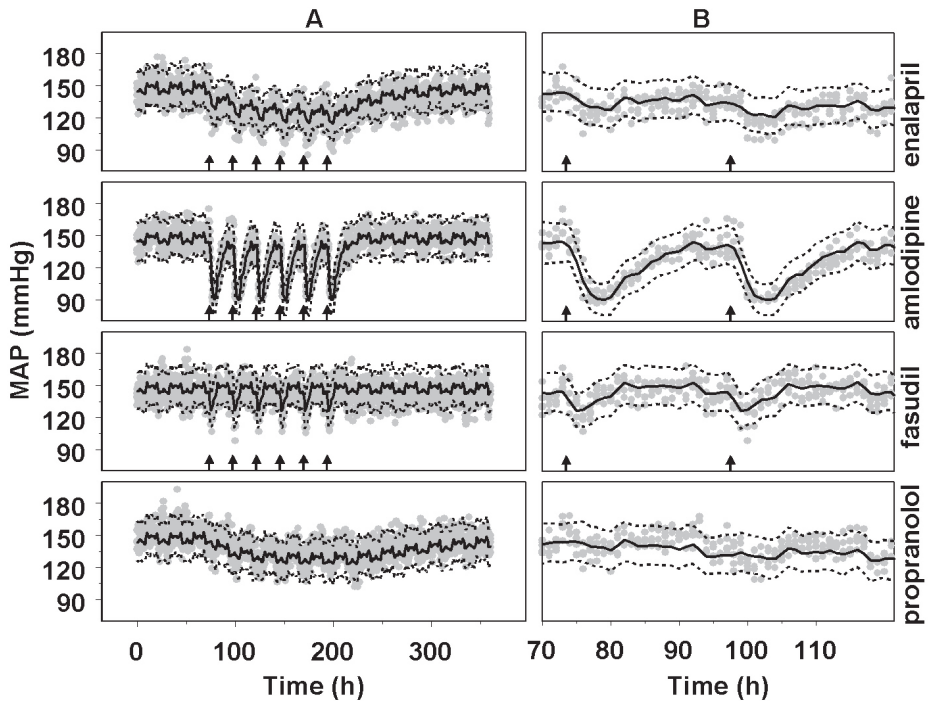

Figure 3: Visual predictive check of the description of the data from the repeated dosing Study 1 by the developed drug-independent CVS model. A) Full time scale; B) Expansion of the first two administrations of each drug. *The grey dots represent the observations in SHR after administration of enalapril (30 mg/kg po) and amlodipine (10 mg/kg p.o.), fasudil (30 mg/kg p.o.) and propranolol (1 mg/mL in drinking water); N=5 SHR/drug. The continuous lines represent the predicted median and the dashed lines represent the predicted lower and upper limit of the 90% prediction interval. The arrows indicate the six daily administrations of each drug.*

In general, the model adequately described the data (Figures 3 and 4). However, for HCTZ the effect of a dose of 1 mg/kg was under-predicted, but the effects of the higher and lower doses of HCTZ were adequately described (Figure 4b). This could indicate that the selected pharmacodynamic model, an E_{max} model with the value of E_{max} fixed to 1, was not optimal. However, this effect model could not be further optimized as the selected dose range was not sufficiently large to cover the complete range from no effect to maximal effect.

All system parameters could be estimated with good precision as all standard errors were less than 50% of the parameter estimates (Table 5). Fixing E_{max} to 1 for amlodipine, fasudil, enalapril and HCTZ did not have a significant influence on the estimates of the system parameters (results shown for amlodipine after fixing the E_{max} of amlodipine to

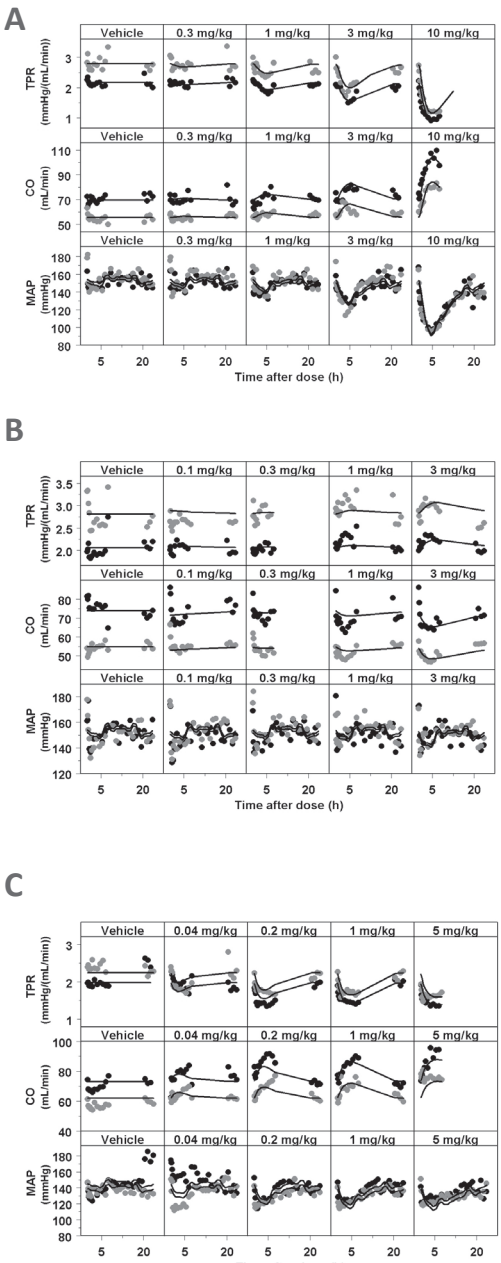

Figure 4: Description of the effects of amlodipine (plot A), HCTZ (plot B) and prazosin (plot C) on cardiac output (CO), total peripheral resistance (TPR) and mean arterial pressure (MAP) by the developed drug-independent CVS model. Data are from Study 2, in which vehicle and a different dose of amlodipine (0.3, 1, 3 and 10 mg/kg p.o.), HCTZ (0.1, 0.3, 1 and 3 mg/kg p.o.) or prazosin (0.04, 0.2, 1 and 5 mg/kg p.o.) was administered on separate days. *The grey and black dots represent the observations of two different rats. The continuous lines represent the individual prediction by the developed drug-independent CVS model after administering amlodipine.*

Table 5: The system parameter values from the drug-independent model to describe the CVS.

Parameter	Value	SE	CV	LLCI	ULCI	*Value when E_{max} of amlodipine was fixed to 0.8 instead of 1*
BSL_TPR (mmHG/(mL/min)	2.32	0.132	5.69	2.06	2.58	*2.32*
BSL_MAP (mmHG)	147	1.38	0.939	144	150	*147*
kout_CO (1/h)	99 FIXED					
kout_TPR (1/h)	0.260	0.129	49.6	0.00716	0.513	*0.308*
SL1 (1/mmHG)	0.00378	0.000148	3.92	0.00349	0.00407	*0.00382*
SL2 (1/mmHG)	0.00492	0.00101	20.5	0.00294	0.00690	*0.00468*

SE: Standard error of parameter estimate

CV: Coefficient of variation

LLCI: Lower limit of 95 % confidence interval

ULCI: Upper limit of 95 % confidence interval

Table 6: The drug-dependent parameter values estimated by the drug-independent model to describe the CVS

Parameter	Value	SE	CV	LLCI	ULCI
Amlodipine					
E_{max}	1 fixed				
IC_{50} (ng/mL)	185	26.2	14.2	134	236
Fasudil					
E_{max}	1 fixed				
IC_{50} (ng/mL)	321	60.3	18.8	203	439
Propanolol					
E_{max}	0.335	0.0624	18.6	0.213	0.457
IC_{50} (ng/mL)	9.82	3.8	38.7	2.37	17.3
Enalapril					
E_{max}	1 fixed				
IC_{50} (ng/mL)	2410	373	15.5	1679	3141
HCTZ					
E_{max}	1 fixed				
IC_{50} (ng/mL)	12300	780	6.34	10771	13829
Prazosin					
E_{max}	0.213	0.0158	7.42	0.182	0.244
IC_{50} (ng/mL)	0.133	0.146	109.8	-0.15	0.4

SE: Standard error of parameter estimate

CV: Coefficient of variation

LLCI: Lower limit of 95 % confidence interval

ULCI: Upper limit of 95 % confidence interval

the arbitrarily selected value of 0.8 (instead of 1) in Table 5). In addition, all drug-specific parameters could be estimated with good precision, except for the EC_{50} of prazosin (CV: 110%) (Table 6). For this compound EC_{50} and E_{max} were estimated simultaneously. Fixing E_{max} to 1, as was done for four other compounds, resulted in a more precise estimate of the EC_{50}, but the goodness of fit was less good as indicated by a significant increase in the MVOF. All correlations between system-specific parameters were less that 0.95, except for the correlation between k_{out_TPR} and *FB2* (-0.984).

Influence of the selection of compounds on the system-parameters

None of the parameters changed significantly when data of one of the six compounds were selectively omitted with the exception of the value of the parameter *FB1*, which was

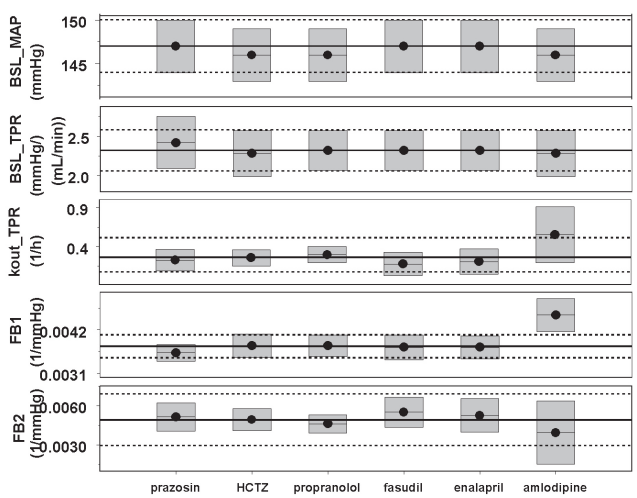

Figure 5: Evaluation of drug-independency of the developed CVS model

Six different compounds, prazosin, HCTZ, propranolol, fasudil, enalapril and amlodipine, were used to estimate the system parameters of the developed CVS model. To determine if the system parameters were truly drug-independent the model was re-evaluated omitting the different compounds one by one. *The continuous black lines represent the parameter estimates of the model including all compounds and the dashed lines represent the 90% confidence intervals around these parameter estimates. The black lines with a black dot and the grey boxes represent the parameter estimates and the 90% confidence intervals around these parameter estimates after omitting one of the compounds. When the grey boxes overlap with the area between the dotted lines, parameters are not significantly different and the model is independent of that compound. Therefore, the parameter estimate of FB1 is dependent on the presence of the amlodipine data.*

found to be dependent on the presence of the amlodipine data (Figure 5). *FB1* changed from 0.00379 (CI: 0.00348-0.00410) to 0.00454 (CI: 0.00418-0.00490) 1/mmHg.

System properties

Clear differences were found between the signature profiles of MAP, CO and TPR after simulating drug effects on CO and TPR. It was found that an increase in MAP can only be obtained by stimulating CO or TPR, and not by an overshoot of the feedback. Specifically, the simulation showed that inhibiting CO or TPR always results in a decrease in MAP, which demonstrates that feedback cannot be stronger than the primary effect (Figure 6).

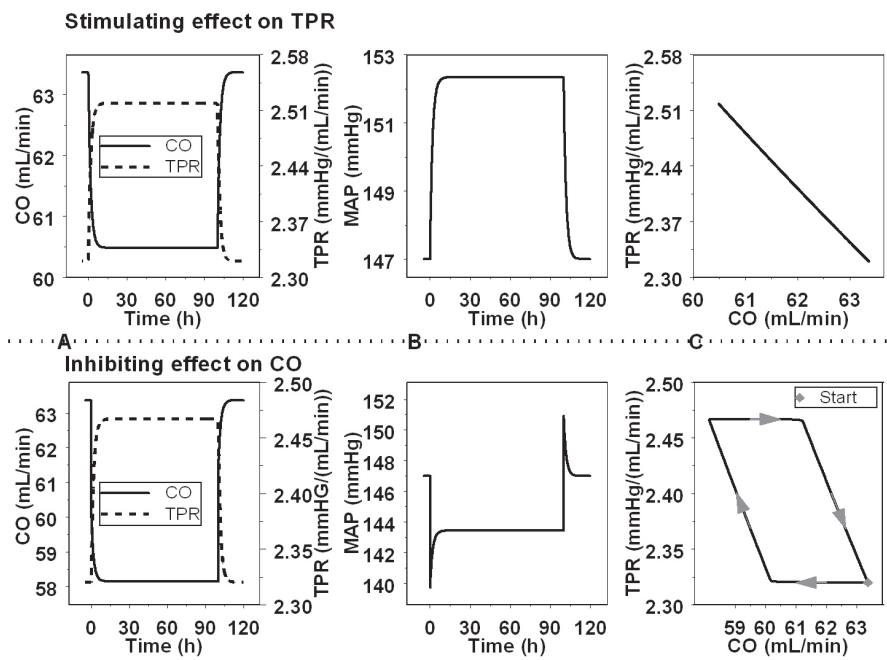

Figure 6: System properties of the CVS

The system properties of the CVS were investigated by simulating the response on CO, TPR and MAP after stimulating TPR (upper panel) or inhibiting CO (lower panel). Both perturbations result in visually comparable effects on CO and TPR (plot A). However, the response on MAP is in the opposite direction (plot B) indicating that the model can be used to identify the site of action. In addition, the hysteresis plot shows that an effect on TPR results in an immediate response on CO as a result of feedback, whereas, an effect on CO results in a delayed response on TPR as a result of feedback (plot C).

In addition, the delay in response was longer when the drug effect was on CO as compared to on TPR (Figure 6c).

Discussion

A mechanism-based PKPD model was developed to describe drug effects on the interrelationship between MAP, CO and TPR using data from preclinical experiments with a training set of six compounds with diverse effects on BP. Several models that describe the physiology of the CVS in great detail have been published, such as the Guyton and Coleman model (Guyton *et al.*, 1972), which has provided the basis for the understanding of long-term BP control (Montani and Van Vliet, 2009). However, to date no models exist that integrate a quantitative description of the physiology of the CVS and the effect of cardiovascular drugs on the relationship between MAP, CO and TPR except for a model that was postulated by Francheteau et al. (Francheteau *et al.*, 1993). This model provides a description of the effect of dihydropyridine drugs on the relationship between MAP, CO and TPR. As several key model parameters of the Francheteau model were not identifiable this is not a truly mechanism-based model in the sense that drug- and system-specific properties were indistinguishable. An important feature of a mechanism-based PKPD model is that both the drug-specific and the system-specific model parameters are identifiable and quantifiable on datasets from preclinical or clinical studies (Danhof *et al.*, 2007). This enables an adequate prediction of cardiovascular drug effects and becomes especially relevant when the interest is to also understand the variation between biological systems (i.e., between species) or between individuals (Danhof *et al.*, 2007). Therefore, the developed model is the first mechanism-based model that can be applied to describe the effect of cardiovascular drugs with different MoA's on the interrelationship between MAP, CO and TPR.

The developed model was based on a number of assumptions. One assumption was that only taking the primary/direct effects of the compounds on either CO or TPR into consideration was sufficient for identifying the system. For compounds like amlodipine and fasudil this assumption can be justified, since these compounds primarily influence TPR. The change in CO, which is observed after administration of these compounds, is thought to be a secondary effect, which is triggered by the feedback mechanisms of the CVS. For compounds like enalapril and propranolol, the MoA is less clear as these compounds influence both CO and TPR albeit with different magnitudes and on different timescales (Table 1). Since the aim of this research was to develop a drug-independent model to describe the functioning of CVS, an adequate description of the system, based on all drug effects,

was considered more important than the best possible description of the individual drug effects of the different compounds.

Another assumption was that all compounds influence the production rates rather than the dissipation rates of CO or TPR. This assumption was based on the MoA of the different compounds. The compounds that have a primary effect on TPR all influence smooth muscle cell contraction rather than causing relaxation. Therefore, assuming that contraction is equivalent to production, modeling of the drug effect on the production rather than the dissipation rate makes mechanistically sense. The two compounds that influence CO, HCTZ and propranolol, have quite different MoA's (Table 1). HCTZ, a diuretic, decreases ventricular stroke volume by decreasing cardiac filling. On the other hand, propranolol reduces sympathetically mediated stimulation of left ventricular contractility and heart rate. Therefore, from a mechanistic point of view, both compounds are thought to also influence the production rather than the dissipation rates. As the MoA of HCTZ and propranolol are quite different, it might be expected that the delay in response, as reflected by k_{out_CO}, would be different for these compounds. However, for both compounds, this delay was too short to quantify with good precision. Therefore, both the effects of HCTZ and propranolol could be adequately described by the model with k_{out_CO} fixed to a high value of 99 1/h. Although k_{out_CO} could not be quantified, the data did contain information about the rate of change in CO being high as fixing this parameter to a lower value resulted in bias in the description of the HCTZ and propranolol data (results not shown). The exact value of k_{out_CO} is only relevant when the interest is in the effect on shorter time scales than investigated in the current studies, i.e. seconds instead of minutes or hours. In addition, in theory, adding one or more compounds with an effect on the dissipation rate would provide additional information for identification of the system parameters. However, from a mechanistic point of view it is difficult to find compounds with an effect on the k_{out} of CO or TPR. For example, enalapril influences the k_{out} of angiotensin I as it inhibits angiotensin-I-converting enzyme. This effect however translates into an inhibition of the kin of angiotensin II which in turn leads to vasodilation. The current model therefore describes the effect of enalapril on the K_{in} of TPR. Moreover, from a data driven point of view including compounds with an effect on k_{out} will only add additional information for quantification of the system parameters when the selected dose range is large enough to reach the maximum effect (Sharma and Jusko, 1996; 1998). In *in vivo* investigations however attainment of the maximum drug effect is not always feasible for safety reasons. Moreover, in situations where rapid adaptation occurs, it may be impossible experimentally to reach the E_{max} (Porchet *et al.*, 1988). An interesting feature of the developed model is that it can be extended to more detailed levels without having to change the structure of the model. For example, the system can be described in more detail by parsing CO into

heart rate and stroke volume. In addition, including more information on the different feedback mechanisms could lead to a model that distinguishes the effects of different classes of antihypertensive drugs in more detail. The feedback mechanisms currently included in the model are likely to reflect the acute compensatory mechanisms (such as the baroreceptor reflex) better than the long term compensatory mechanisms (such as the renin-angiotensin-aldosterone system (RAAS)) as the baroreflex system is active within minutes to hours to days whereas the RAAS is active within hours to days/weeks. To evaluate if the model is predictive for long-term effects on the CVS long-term studies of days or weeks with CO measurements are required.

The assumptions made regarding the use of PK models derived from published results may have a large impact on the PK profiles. Therefore, the PK models were descriptive and the PK and drug-specific PD parameters may not represent “true” values. Therefore, these estimates should only be interpreted in the context of this model. This was considered acceptable as system-specific parameters, which are of primary interest in this research, are considerably less sensitive to changes in PK compared to drug-specific parameters. This is explained by the fact that drug-specific parameters are directly dependent on the PK of a specific drug, whereas the values of system-specific parameters are determined by the data of all compounds.

Beforehand it was hypothesized that two aspects of the experimental design were pivotal to successfully quantify the parameters of the CVS model: i) the selection of the training set of compounds to challenge the functioning of the CVS and ii) measuring both MAP and CO during the on- and offset phases of the drug effects. The correlations between some drug- and system-specific parameters were high (results not shown). However, evaluating if the model was indeed drug independent has demonstrated that the selected combination of compounds was adequate to develop a drug-independent model as only the parameter *FB1*, i.e. 1 of the 5 system-specific parameters, changed when the data of 1 of the 6 compounds (amlodipine) were omitted (Figure 5). To evaluate the importance of measuring both MAP and CO during the on- and offset phases of the drug effects, a retrospective sensitivity analysis was performed, using the parameter estimates of the developed model (Appendix). This sensitivity analysis demonstrated that measuring both MAP and CO during the on- and offset phase provided the pertinent information to quantify the system parameters. This is in agreement with the good precision of the estimates of all system-specific parameters. However, the values of k_{out_TPR} and *FB2* were strongly correlated (-0.984) indicating that there was not enough information to estimate both parameters independently. This was confirmed by the sensitivity analysis, which showed that both parameters are most sensitive to the data collected during approximately the

same period after drug administration and during the offset phase of the drug effects for compounds influencing TPR (Appendix: Figure A). For compounds that influence CO these peaks are more distinct (results not shown), which indicates that the information to estimate these parameters independently was mainly provided by the compounds with an effect on CO. In the current research, only two compounds were included with a primary effect on CO, i.e., propranolol and HCTZ, and CO was measured only after administration of HCTZ. To distinguish these parameters, detailed MAP and CO measurements from more compounds with an influence on CO would be required. This should be taken into consideration when the model is applied for simulation purposes. Measuring CO provided insight into the magnitude of the counteracting effects on TPR and CO underlying the effect on BP. Since MAP is the primary regulated hemodynamic variable, drug effects on TPR and CO were disproportionately greater than those reflected by MAP alone. This indicates that a small observed pharmacologic effect on MAP may mask much larger therapeutic benefits or, conversely, an increased risk of cardiovascular disease. Based on estimates of the residual error, the model is qualified to distinguish changes in MAP, CO and TPR larger than 7.6 mmHg, 4.3 ml/min and 0.5 mmHg/(ml/min), respectively, from noise. This indicates that the model can be used to identify clinically relevant changes in BP. In conclusion, the rigorous experimental design was adequate to provide the data to describe the interrelationship between MAP, CO and TPR in a quantitative and mechanism-based manner.

The developed CVS model can be applied to estimate drug-specific parameters for new compounds, but this requires accurate and precise description of the pharmacokinetics. Recently, novel approaches have been proposed to accurately characterize pharmacokinetics without influencing the pharmacodynamics in pre-clinical PKPD investigations, e.g. the PK can be measured after completion of the pharmacodynamic part of the study (Bender *et al.*, 2009) or the PK and PD can be measured on different days during the study (Viberg *et al.*, 2012). In addition, the developed model can be applied to identify the site of action of new compounds influencing MAP through an unknown MoA, as it was shown in a simulation experiment that distinct differences exist between the signature profiles of compounds with an effect on CO or TPR (Figure 6). In this context, the developed model provides key insights to support drug development, i.e. to learn about the underlying MoA of compounds with desired or undesired effects on BP. The model can also be applied to test hypotheses, e.g., hypotheses on multiple sites of action can be evaluated by including drug-effects on multiple parameters in the model. It should be noted that the identified set of system parameters is specific for spontaneously hypertensive rats. Drug effects on MAP, CO and TPR may vary considerably in other (normotensive) rat strains due to physiological differences (Pinto *et al.*, 1998). Consequently, applications of the

developed model, using the identified set of system parameters, are limited to this rat strain. However, an advantage of a mechanism-based model is that it allows accurate extrapolation between different rat strains and from one species to another (Danhof *et al.*, 2008; Ploeger, 2009) as the structure of the model is expected to be the same in all species. Therefore, an ultimate application of the developed drug-independent model would be to facilitate the anticipation of the clinical response based on preclinical data for newly developed compounds. Before our model can be applied for that purpose, the predictability of long-term blood pressure effect should be evaluated and the model should be scaled to human and validated on human MAP and CO measurements.

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Abbreviations

Amp	Amplitude
BP	Blood pressure
BSL_CO	Baseline value of cardiac output
BSL_MAP	Baseline value of mean arterial pressure
BSL_TPR	Baseline value of total peripheral resistance
C	drug concentration in plasma
CO	Cardiac output
CVS	Cardiovascular system
E_{max}	Maximum effect
EC_{50}	Concentration resulting in a half-maximal effect
FB1	negative feedback of mean arterial pressure on cardiac output
FB2	negative feedback of mean arterial pressure on total peripheral resistance
HCTZ	Hydrochlorothiazide
HOR	Horizontal displacement
IIV	Inter-individual variability
K_{in_CO}	Zero-order production rate constant of cardiac output
K_{in_TPR}	Zero-order production rate constant of total peripheral resistance
k_{out_CO}	First-order dissipation rate constant of cardiac output
k_{out_TPR}	First-order dissipation rate constant of total peripheral resistance
MAP	Mean arterial pressure
MC	Methylcellulose
MoA	Mechanisms of action
MVOF	Minimum value of the objective function
PD	Pharmacodynamics
PK	Pharmacokinetics
PKPD	Pharmacokinetic-pharmacodynamic
RAAS	Renin-angiotensin-aldosterone system
SHR	Spontaneously hypertensive rats
T	Time
TPR	Total peripheral resistance

Appendix: Sensitivity analysis, evaluation of the experimental design

An adequate experimental design was thought to be pivotal to distinguish drug- from system-specific parameters in this investigation. By showing how the dynamic behavior of the system responds to changes in parameter values, a sensitivity analysis enables identification of the part of the experimental protocol that provides the pertinent information to quantify the parameters and to distinguish one parameter from another. Using the parameter estimates of the developed model, a retrospective parameter sensitivity analysis was performed in Berkeley Madonna (version 8.3.5, Berkeley Madonna Inc., University of California) to determine how "sensitive" the developed model is to changes in the values of the parameters of the model.

First a simulation was performed with all system parameters fixed at their estimated values ($S(t,x_0)$), while assuming an inhibiting drug effect on TPR during a constant drug infusion of 100 h to ensure that the drug effect is in steady state. Subsequently, simulations were performed after 0.1% increments in the system parameters (0.1% is the standard in Berkeley Madonna) ($S(t,x)$). Finally, for each parameter, the sensitivity ($S(t)$) was calculated according to Equation A.1.

$$S(t) = x_0 \frac{\partial S(t,x)}{\partial x} \cong \frac{S(t,x) - S(t,x_0)}{(x - x_0)/x_0} = \frac{S(t,x) - S(t,x_0)}{0.1\%} \quad \text{(A.1)}$$

The sensitivity ($S(t)$) in change from baseline for MAP, CO and TPR was evaluated for all system parameters (Figure A.1). This figure shows that the on- and offset phases of the drug effect contained complementary information as in both phases the peaks of the values of the different parameters of the pharmacodynamic system (BSL_TPR, BSL_MAP, k_{out_TPR}, FB1 and FB2) occurred at different time points relative to each other. In addition, the three biomarkers of the CVS, MAP, CO and TPR also contained complementary information regarding the dynamics of the system. For example, the peaks of the two feedback parameters FB1 and FB2 occurred almost simultaneously when examining the sensitivity in MAP, whereas when looking at the sensitivity in CO and TPR the peaks occurred relatively later. Therefore, measuring CO provided the pertinent information to distinguish these parameters.

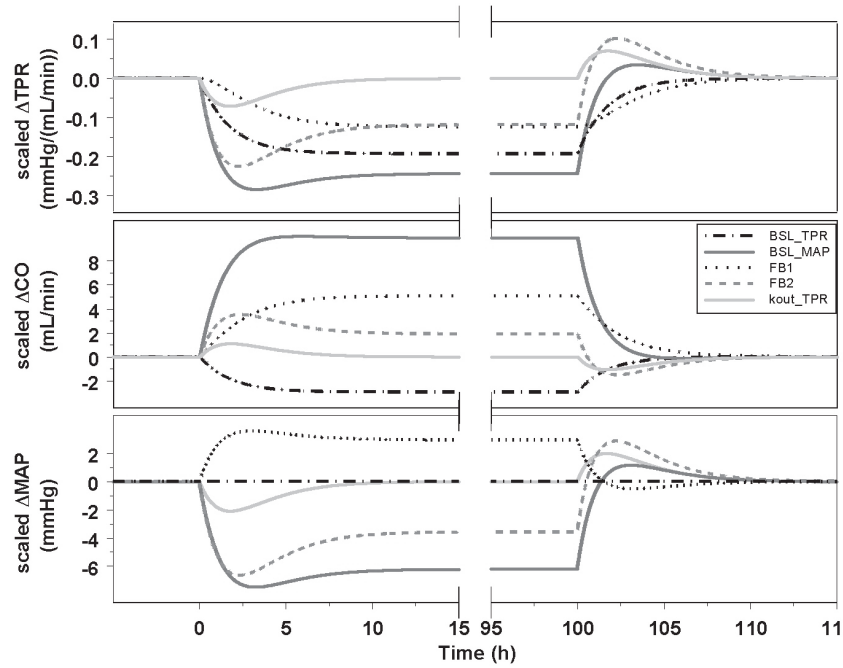

Figure A: Sensitivity analysis

Influence of a 0.1% increase in the values of the system parameters of the drug-independent model (BSL_TPR, BSL_MAP, k_{out_TPR}, FB1 and FB2) on the dynamic behavior of the CVS parameters MAP, CO and TPR. In this sensitivity analysis an inhibiting drug effect (an on/off response; constant infusion during 100 h)) on TPR was simulated.