# Towards predictive cardiovascular safety : a systems pharmacology approach

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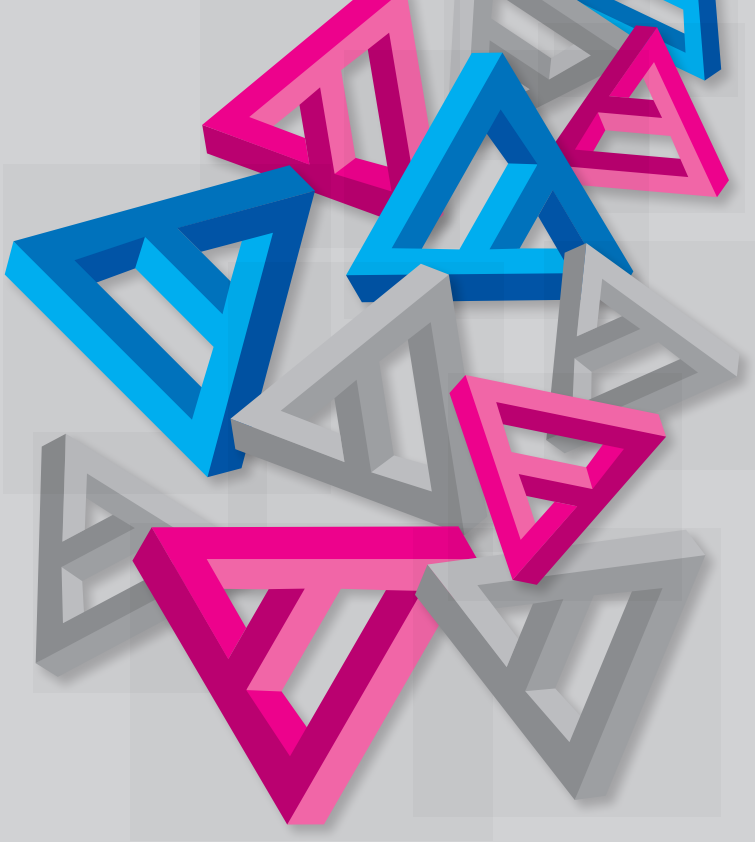

# CHAPTER 7

A systems pharmacology approach to the prediction of cardiovascular side effects in man - Summary, conclusions & perspectives

## Introduction and objectives

Cardiovascular safety issues related to changes in blood pressure, arise frequently in drug development. In this thesis, a systems pharmacology model is described which allows predicting hemodynamic changes in man on the basis of information from pre-clinical studies. The proposed model is based on well-established principles of the regulation of blood-pressure. It is well-known that mean arterial pressure (MAP) equals the product of cardiac output (CO) and total peripheral resistance (TPR) and that CO equals the product of HR and stroke volume (SV). However, the effects of drugs on this interrelationship have not been analyzed in a mechanism-based and quantitative manner. A pertinent feature of mechanism-based pharmacokinetic-pharmacodynamic (PKPD) models is the distinction between drug and biological-system specific properties for the characterization of *in vivo* drug effects (Danhof *et al.*, 2007; Ploeger *et al.*, 2009*)*. In this regard, drug-specific parameters describe the interaction between the drug and the biological system in terms of target affinity and target activation, whereas system-specific parameters describe the dynamics of the biological system. This separation enables prediction and extrapolation of treatment effects to biological systems other than the systems that have been studied (e.g. the translation from laboratory animals to humans) (Danhof *et al.*, 2007). Typically, mechanism-based and mechanistic PKPD models are based on an analysis of the causal path between drug exposure and response. More recently the concept of systems pharmacology has been introduced. With systems pharmacology models the complexity is increased by focusing on networks and the interactions between different components of the network. A systems pharmacology modeling approach is uniquely suited to quantify drug effects on the interrelationship between MAP, CO, HR, SV and TPR (**Chapter 1**).



The objectives of the investigations described in this thesis were 1) to establish a systems pharmacology model to characterize the effects of drugs with different mechanisms of action (MoA's) on the interrelationship between MAP, CO, HR, SV and TPR in a quantitative manner and 2) to apply the model to the quantification of the cardiovascular effects of the sphingosine 1-phosphate (S1P) receptor modulator fingolimod. In this chapter, the results of the investigations described in this thesis are reviewed and the perspectives and conclusions are presented.

## Development of a systems pharmacology model to characterize the CVS

In **Chapter 3**, a systems pharmacology model was proposed to characterize the CVS in hypertensive rats. This system pharmacology model is based on the interrelationship between MAP, CO and TPR. The effects of a prototype set of cardiovascular compounds

with different mechanisms of action were characterized in chronically instrumented spontaneously hypertensive rats (SHR). Beforehand it was hypothesized that two aspects of the experimental design were pivotal to successfully quantify the parameters of the basic CVS model: i) the selection of the prototype set of compounds affecting the functioning of the CVS and ii) measuring both MAP and CO during the on- and offset phases of the drug effects. By selecting a range of cardiovascular drugs with well described, but different target sites and with different profiles of the time courses of the effects, all parameters of the systems pharmacology model could be estimated with good precision. Moreover, system-specific parameters could be distinguished from drug-specific parameters indicating that the developed model is drug-independent. The measurement of CO, was pivotal to identify all parameters of the basic systems pharmacology model as this provide the information to distinguish between changes in CO and TPR. To be able to measure drug effects on MAP and CO, rats were surgically instrumented with both an ascending aortic flow

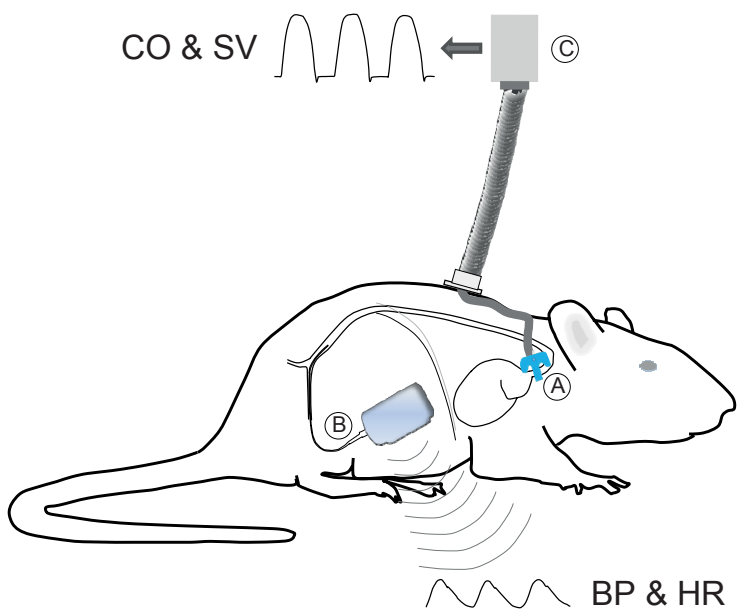


**Figure 1:** Experimental animal instrumentation. Rats were surgically instrumented with both an ascending aortic flow probe (A) and a femoral arterial catheter/radiotransmitter (B). CO was measured by connecting the flow probe to the flow meter via a cable and electrical swivel (C), which allowed the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

probe and a femoral arterial catheter/radiotransmitter (Figure 1). Overall, the rigorous experimental design provided the data to describe the interrelationship between MAP, CO and TPR in a quantitative and mechanism-based manner. The basic CVS model can be applied to elucidate the site of action of new compounds which affect MAP through a still unknown MoA.

A limitation of this model is that measuring CO has not been integrated into daily practice due to the challenges associated with the implantation of aortic flow probes. Therefore, in **Chapter 4** the basic CVS model was extended by parsing CO into HR and SV (Figure

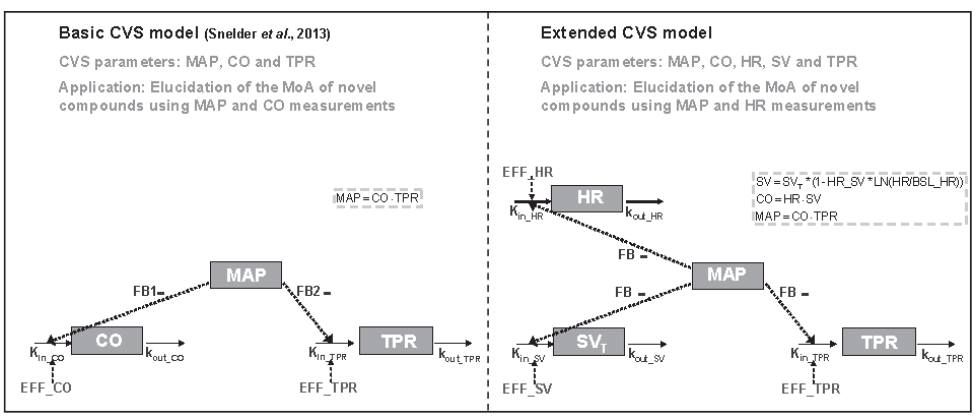


**Figure 2:** Comparison between the basic CVS model to characterize drug effects on the interrelationship between MAP, CO and TPR and the extended CVS model to characterize drug effects on the interrelationship between MAP, CO, HR, SV and TPR

***Basic CVS model (Chapter 3):*** *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.*

***Extended CVS model (Chapter 4)****: MAP equals the product of CO and TPR (MAP=CO*TPR) and CO equals the product of HR and SV (CO=HR*SV)). SV is influenced by indirect feedback through MAP ($SV_T$) and by HR through a direct inverse log-linear relationship, where HR_SV represents the magnitude of this direct effect. Effects on HR, SV and TPR are described by three linked turnover equations. When MAP increases as a result of a stimulating effect on HR, SV or TPR, the values of HR, SV and TPR will decrease as a result of the action of the different feedback mechanisms regulating the CVS. The magnitude of feedback on HR, SV and TPR is represented by FB. Kin_CO, Kin_HR, Kin_SV and Kin_TPR represent the zero-order production rate constants and kout_CO, kout_HR, kout_SV and kout_TPR represent the first-order dissipation rate constants*

2) with the aim to 1) characterize drug effects on the interrelationship between HR and MAP, which are both important variables in the safety evaluation of novel drugs and 2) investigate if the MoA of new compounds can be elucidated using HR and MAP measurements only.

The behavior of the extended CVS model was evaluated by simulating the changes in MAP, CO, HR, SV and TPR after triggering the model by inhibiting HR, SV or TPR with a hypothetical compound after a single oral dose (Figure 3). The characteristic profiles of the time-course of the drug effect on MAP, CO, HR, SV and TPR are referred to as signature profiles. For the different variables distinctly different profiles were observed. From these simulations it can be concluded that, even if CO is not measured, it is likely that the extended CVS model can be used to elucidate the site of action of novel compounds with a single MoA (i.e., one site of action). In summary, when the nature (i.e. an increase or decrease) of the drug induced change in HR and MAP is the same, it is likely that the primary effect is on HR. When HR and MAP change in opposite directions, i.e. an increase in HR and a decrease in MAP or *vice versa*, the primary effect of the drug is expected to be on SV or TPR. Effects on SV and TPR can be distinguished by the delay between the perturbation and the effect on MAP, i.e. a long delay indicates that the primary effect is on SV and a short delay indicates that the primary effect is on TPR. Next to MAP, HR is an important parameter in the safety evaluation of novel drugs for a wide variety of disorders (Sudano *et al.*, 2012; Gasparyan *et al.*, 2012; Cardinale *et al.*, 2013; Guth, 2007). Although MAP and HR are usually measured simultaneously, it is common practice to quantify drug effects on these hemodynamic parameters independently resulting in two separate dose/concentration-effect relationships. However, this approach disregards the interrelationship between MAP and HR. Therefore, an additional advantage of extending the basic CVS model by parsing CO into HR and SV is that drug effects on MAP, CO, HR, SV and TPR can be characterized simultaneously yielding a single unique set of parameter estimates. Furthermore, the basic CVS model was extended to a more detailed level by quantifying differences in blood pressure regulation between normotensive and hypertensive rats. As there are pronounced differences in MAP regulation between hypertensive and normotensive rats (Pinto *et al.*, 1998) the magnitude of the effect of cardiovascular drugs on the different hemodynamic endpoints varies considerably between strains. Therefore, the basic CVS model is not directly applicable to data from normotensive rats. This is a major drawback especially for drug safety evaluations, which are usually conducted in normotensive rats. As the ultimate aim of the proposed quantitative pharmacology model is to predict clinical responses to novel pharmacologic agents, it is pivotal that the CVS model is applicable to both normotensive and hypertensive rats. The baseline parameters were found to differ per strain with a higher baseline value of MAP and a lower baseline value

of CO for hypertensive as compared to normotensive rats, whereas the baseline value for HR did not significantly differ between the strains. The baseline values of SV and TPR were derived from these parameters, resulting in a lower baseline value of SV and a higher baseline value of TPR for hypertensive as compared to normotensive rats. In addition, feedback was found to decrease with the individual value of the baseline of MAP. Overall, the feedback was about 2-fold higher in normotensive rats as compared to hypertensive rats indicating impaired blood pressure regulation in hypertensive rats. In conclusion, a systems pharmacology model, characterizing the interrelationship between MAP, CO,

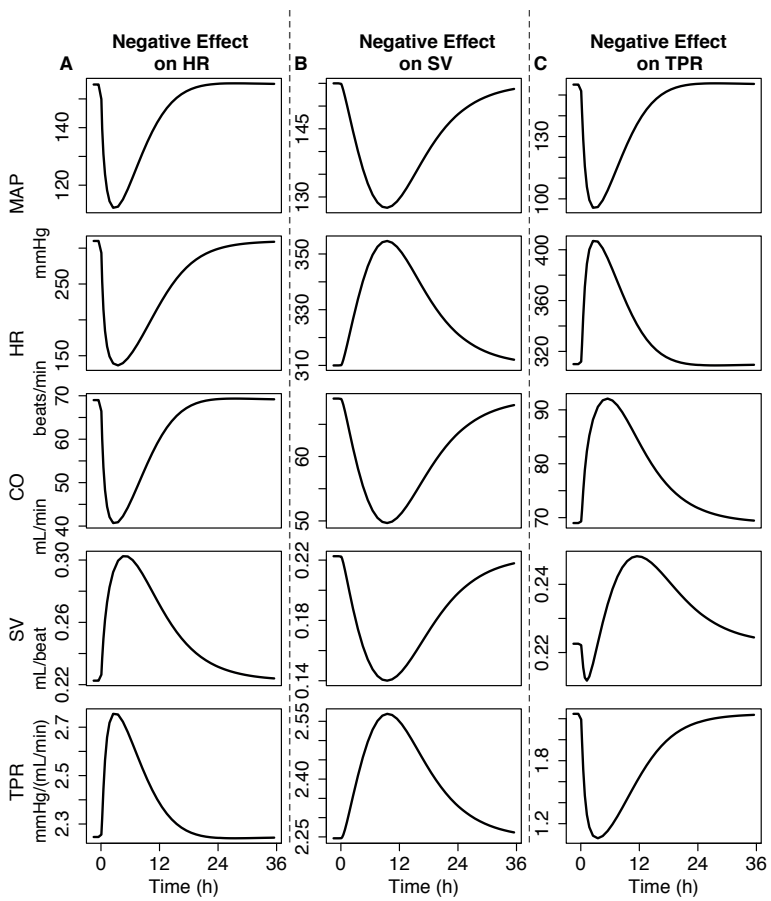


**Figure 3:** System properties of the CVS

*The system properties of the CVS were investigated by simulating the response on MAP, CO, HR and TPR after inhibiting HR (A), SV (B) or TPR (C). Inhibiting HR, SV or TPR always results in a decrease in MAP, which demonstrates that feedback cannot be stronger than the primary effect. In addition, the delay in response on MAP was longer when the drug effect was on SV as compared to TPR.*

HR, SV and TPR, was obtained in hypertensive and normotensive rats. The extended CVS model can be used to quantify the dynamic changes in the CVS and elucidate the MoA for novel compounds using HR and MAP measurements only. 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. An ultimate application of the extended CVS model would be to facilitate the anticipation of the clinical response based on preclinical data for newly developed compounds. Before the extended CVS model can be applied for that purpose, the system-specific parameters of the model should be scaled to human and validated on human MAP, HR and CO measurements as detailed in the section "Towards characterization and prediction of cardiovascular drug effects in humans".

## Application of the systems pharmacology model to S1P receptor agonists

In humans, S1P receptor modulators, which are effective in the treatment of multiple sclerosis (Cohen *et al.*, 2010; Gergely *et al.*, 2012), are associated with cardiovascular side effects. More specifically, at the therapeutic dose of 0.5 mg, a transient decrease in HR (8 beats per minute with attenuation after 6 hours) and a small increase in MAP (1-2 mmHg after 2 months) were observed after administration of fingolimod (Kappos *et al.*, 2010). In addition, at the supra-therapeutic doses of 1.25 and 5 mg, HR was reduced within 6 hours by a mean of 13.8 and 16.6 beats per minute, respectively, returning towards the baseline value with continued treatment. At these doses, MAP was mildly increased by 5-6 mmHg following continuous treatment (Kappos *et al.*, 2006).

The immunosuppressant effects, as well as the cardiovascular side effects, of these compounds are believed to be mediated through the S1P receptor, which complicates the search for novel S1P receptor modulators, which are devoid of cardiovascular side effects. A quantitative understanding of the hemodynamics of these effects is important as it may constitute a basis for the selection of new compounds with an improved safety profile. Moreover, it may provide insights in the possibility to prevent and reverse these effects for new S1P receptor modulators by co-administration of other drugs (Kovarik *et al.*, 2008), or to design dose titration schemes to attenuate these effects (Legangneux *et al.*, 2013). The extended CVS model is uniquely suited to provide a quantitative understanding of these effects as the structure of the model and the values of the parameters describing the model are independent of the drugs that were used to create it and, therefore, the model can be used to quantify the dynamic changes in the CVS for other compounds and thus for fingolimod. A potential application of this model is the prediction of the cardiovascular effects of novel compounds. This requires interfacing the CVS model with a target

binding-activation model. Moreover, the proposed systems pharmacology model may allow prediction of cardiovascular effects of S1P receptor modulators in humans based on preclinical evaluations. A complicating factor in the translational pharmacology of fingolimod and, in general S1P receptor modulators, is that they exert their pharmacological effect through their respective phosphate metabolites, which are formed by the enzyme sphingosine kinase (S1PHK). At first, the pharmacokinetics (PK) of fingolimod and its active metabolite fingolimod-phosphate (fingolimod-P) were characterized in rats and humans. Since large inter-species differences exist in the S1PHK enzyme tissue distribution and enzyme activity, it is anticipated that the PK of S1PHK substrates in rats may not be scalable to humans using a standard allometric scaling approach. In **Chapter 5**, a semi-mechanistic PK model for the inter-conversion of S1PHK substrates and their respective phosphates in rats and humans was proposed. A specific aim was to determine whether species specific differences in the *ex vivo* rate of phosphorylation in blood platelets are representative for the differences in the phosphorylation in general and might therefore constitute a basis for pharmacokinetic scaling. To explore this, data on the time course of fingolimod and fingolimod-P blood concentrations in rats following intravenous administration of fingolimod and fingolimod-P as well as following oral administration of fingolimod were analyzed in conjunction with data on the *ex vivo* inter-conversion and blood-plasma distribution in rat blood. Separately, data from two healthy volunteer studies, in which fingolimod was administered orally in doses of 0.5, 1.25 and 5 mg once daily, were simultaneously analyzed with data on the *ex vivo* inter-conversion and blood-plasma distribution in human blood. Overall, the PK of fingolimod and fingolimod-P in rats and humans was adequately characterized by a semi-mechanistic model in which phosphorylation of fingolimod occurred pre-systemically during first-pass in the liver and in the platelets (Figure 4). In addition, dephosphorylation occurred in the plasma. Integrating data from the *ex vivo* and *in vivo* studies enabled prediction of fingolimod and fingolimod-P concentrations in plasma rather than blood. This is important because plasma concentrations are more relevant for predicting drug effects as, according to the free drug hypothesis, only drug present in plasma is able to bind to its target. Moreover, inter-species differences in the rate of phosphorylation could be quantified. In humans, phosphorylation of fingolimod in the platelets was 4 times slower compared to rats, whereas the de-phosphorylation rates were comparable in both species. In conclusion, using fingolimod as a paradigm compound, large interspecies differences in the rate of phosphorylation between rats and humans were demonstrated, which cannot be accounted for by allometric scaling. Although, this only partly explained the 12-fold over-prediction of fingolimod-P exposure in humans when applying an allometric scaling approach on the developed rat model, the developed semi-mechanistic PK model constitutes a basis for the prediction of the

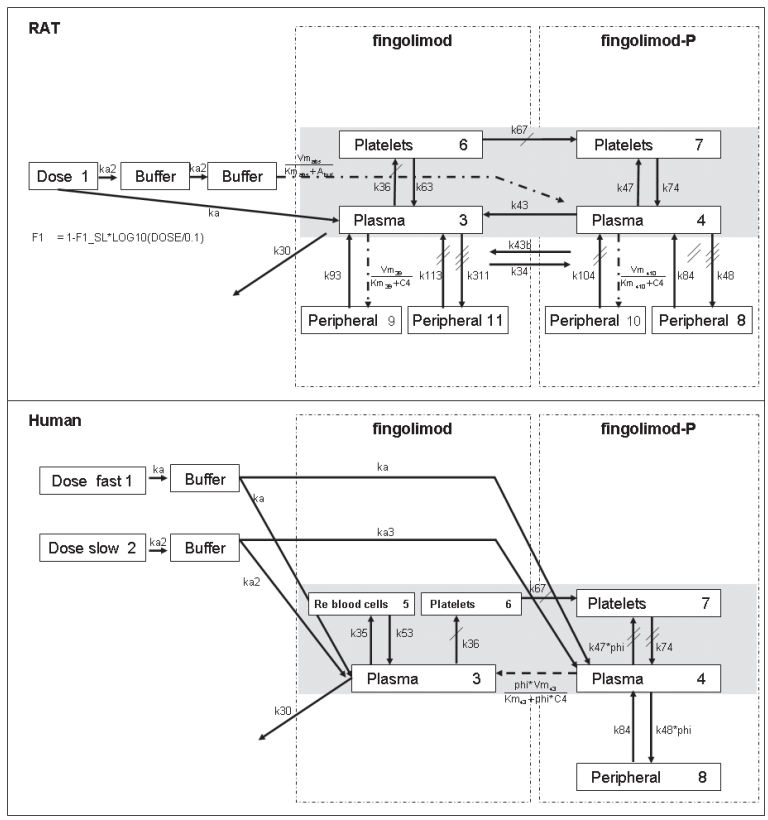


**Figure 4:** Pharmacokinetic models to describe the time course of the fingolimod and fingolimod-P blood concentration in rats and humans.

*The grey area describes the inter-conversion and blood/plasma distribution in isolated blood. Kxy represent the first-order distribution and elimination rate constants. Arrows with an equal number of slashes indicate that these rates are the same. In addition, the dashed lines represent saturable processes.*

concentrations of S1PHK substrates and their phosphorylated metabolites in plasma. However, differences in pre-systemic phosphorylation should also be taken into account.

In **Chapter 6**, the extended CVS model and semi-mechanistic PK model for fingolimod and fingolimod-P in rats were applied to characterize the cardiovascular effects following the administration of fingolimod in normotensive and hypertensive rats. Briefly, data on the concentrations of fingolimimod-P and the changes in various hemodynamic variables were analyzed on the basis of the extended CVS model. In a first step, a model-based

hypothesis testing procedure was followed using the extended CVS model without changing the system-specific parameters.

1) Different hypotheses of the site of action (i.e. HR, SV and TPR) and direction of the effect (i.e., inhibiting or stimulating) were formulated, resulting in 6 possible combinations of effects.
2) For each hypothesis, the model was fitted to the MAP, CO, HR, SV and TPR measurements.
3) It was evaluated which hypothesis resulted in the best description of the data.

It was found that effect of fingolimod-P on the CVS could be described while assuming multiple sites of action, i.e. TPR and HR (Snelder *et al.*, 2013). Overall three different effects were quantified: 1) a fast stimulating effect on TPR, 2) a slow sustained stimulating effect on TPR which is only relevant in hypertensive rats following doses higher than 1 mg/kg and 3) a transient inhibiting effect on HR. In this first step, drug effects were described by empirical models. This provided information on the most plausible sites of action of fingolimod-P, but it also demonstrated that extended CVS model can be applied to quantify the hemodynamics of the effect of fingolimod-P on five different parameters, i.e. MAP, CO, HR, SV and TPR, while assuming only two different sites of action. In a next step, the obtained information on the site of action of fingolimod-P was compared with the available information on the mechanism of action of fingolimod-P. Briefly, at the time of the analysis the following was known about the MoA underlying the effect of fingolimod-P on the CVS.

i. Fingolimod-P influences HR through to binding to the $S1P_1$ receptor. The arterial muscarinic-gated potassium channel IKACH is activated (Koyrakh *et al.*, 2005), which results in a negative chronotropic effect.
ii. Fingolimod-P induces internalization and degradation of the $S1P_1$ receptor (Horga *et al.*, 2010; Mullershausen *et al.*, 2009). As a result fingolimod-P acts a functional antagonist.
iii. Fingolimod-P influences TPR through binding to the $S1P_3$ receptor (Peters and Alewijnse, 2007; Coussin *et al.* 2002*)*. The $S1P_3$ receptor is expressed in vascular smooth muscle cells and regulates the contractility of these cells. As a result fingolimod-P increases peripheral resistance and thus blood pressure.
iv. The major trigger for smooth muscle cell contraction is a rise in intracellular calcium concentration. Whereas the calcium-dependent phase smooth muscle cell contraction is rapid and relatively transient, calcium sensitization produced by agonist stimulation results in as sustained contraction of vascular smooth muscle cells (Watterson *et al.*, 2005), and thus, in a sustained increase in TPR.

Since this information is in line with the elucidated site of action of fingolimod-P the empirical drug-effect models were replaced by models based on receptor theory concepts

for the characterization of target binding and target activation processes, which improved the properties for extrapolation to humans and the prediction of the effects of follow-up compounds (Danhof *et al.*, 2007; Ploeger *et al.*, 2009). As fingolimod-P is an agonist for the S1P receptor competitive interaction between the endogenous agonist, S1P, and fingolimod-P was taken into account for both the effects on HR and TPR. The developed model existed of expressions to describe 1) competitive receptor binding kinetics to the $S1P_1$ receptor (fast effect on HR), 2) $S1P_1$ receptor internalization and degradation (transient nature of the fast effect on HR), 3) competitive receptor binding kinetics to the $S1P_3$ receptor (fast effect on TPR), and 4) $S1P_3$ receptor sensitization (slow effect on TPR) (Figure 5). By characterizing drug effects on HR and TPR, the effects of fingolimod-P on MAP, CO, HR, SV and TPR were adequately described in hypertensive and normotensive rats. The dissociation constants for the effects on TPR ($Kd_{TPR}$; binding to the $S1P_3$ receptor) and HR ($Kd_{HR}$; binding to the $S1P_1$ receptor) were estimated to be 17.7 [confidence interval (CI): 3.74–31.6] and 132 [CI: 68.9–195] nM based on free plasma concentrations, respectively. The $Kd_{TPR}$ is in the same order of magnitude as the *in vitro* binding dissociation constant for binding of fingolimod-P to the human $S1P_3$ receptor reported by Mandale *et al.* of 3.15±1.7 nM (Mandala *et al.*, 2002; calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973)). However, the estimated $Kd_{HR}$ differs from the reported *in vitro* dissociation constants for the binding of fingolimod-P to the human $S1P_1$ receptor of 0.17±0.14 nM (Mandala *et al.*, 2002; calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973)). It should be noted that, *in vivo,* the $S1P_1$ receptor is internalized and degraded. This could confound the estimation of the dissociation constant. Moreover, it should be noted that the reported dissociation constants reflect binding to human $S1P_1$ and $S1P_3$ receptors. Dissociation constants for binding to rat $S1P_1$ and $S1P_3$ receptors are not reported and it is known that large interspecies differences may exist.

Since the developed model was based on receptor theory concepts for the characterization of target binding the model could readily be applied to predict the effect of siponimod, a S1P receptor agonist with different receptor subtype selectivity, on the CVS by correcting the estimated dissociation for fingolimod-P for the molecular weights, the unbound fractions and the ratio of the potencies derived from *in vitro* binding assays. Overall, the effect of siponimod on MAP and HR in rats was adequately predicted by the model (using its *in vitro* receptor binding constants), which indicates that the developed model can be applied to predict the effect of other S1P modulators on the CVS in rats. Ultimately, the proposed system pharmacology model may allow prediction of cardiovascular effects of S1P receptor modulators in humans based on preclinical evaluations of drug effects. However, this requires several steps, which are described in the next section.

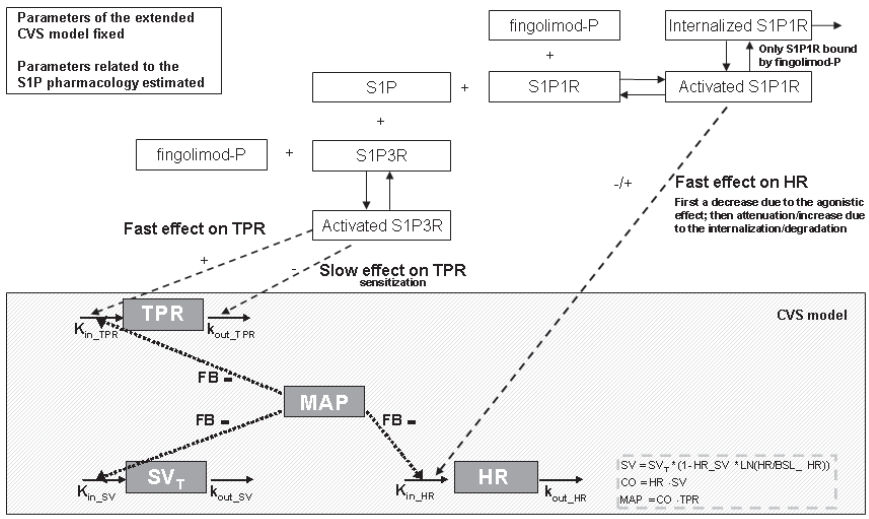


**Figure 5:** Cardiovascular model to describe drug effects on the interrelationship between mean arterial pressure (MAP), cardiac output (CO), heart rate (HR), stroke volume (SV) and total peripheral resistance (TPR) after administration of fingolimod.

***Extended CVS model:*** *Cardiac output (CO) equals the product of HR and SV (CO=HR*SV) and MAP equals the product of CO and TPR (MAP=CO*TPR). SV influenced by indirect feedback through MAP ($SV_T$) and by HR through a direct inverse log-linear relationship, where HR_SV represents the magnitude of this direct effect. Effects on HR, SV and TPR are described by three linked turnover equations. In these equations Kin_HR, Kin_SV and Kin_TPR represent the zero-order production rate constants and kout_HR, kout_SV and kout_TPR represent the first-order dissipation rate constants. When MAP increases as a result of a stimulating effect on HR, SV or TPR, the values of HR, SV and TPR will decrease as a result of the action of the different feedback mechanisms regulating the CVS. In this model the magnitude of feedback on HR, SV and TPR is represented by FB.*

***Effect of fingolimod-P:*** *The primary effect of fingolimod-P is on TPR. The effect on TPR is a combination of a fast stimulating effect and a slowly occurring stimulating effect (sensitization). The effect on HR is initially an inhibiting effect. However, as a result of the receptor internalization this effect rapidly attenuates.*



## Perspectives - Towards characterization and prediction of cardiovascular drug effects in humans

An ultimate application of the developed systems pharmacology model is to predict cardiovascular effects in man on the basis of information from pre-clinical studies for newly developed compounds and, more specifically, for S1P receptor agonists, i.e. follow-up compounds of fingolimod. In this section, first, the inter-species translation of the system-specific CVS model is discussed. Thereafter, the inter-species translation of the effect of S1P receptor agonists is addressed.

### *Translation of the system-specific CVS model*

The extended CVS model consisted of three turnover equations, for HR, SV and TPR respectively, which were linked by negative feedback through MAP (Equation 1).

$$\frac{dHR}{dt} = K_{in\_HR} \cdot (1 + CR_{HR}) \cdot (1 - FB \cdot MAP) - k_{out\_HR} \cdot HR$$
$$\frac{dSV_T}{dt} = K_{in\_SV} \cdot (1 - FB \cdot MAP) - k_{out\_SV} \cdot SV_T$$
$$\frac{dTPR}{dt} = K_{in\_TPR} \cdot (1 + CR_{TPR}) \cdot (1 - FB \cdot MAP) - k_{out\_TPR} \cdot TPR \quad (1)$$

$$SV = SV^{*} \cdot (1 - HR\_SV \cdot LN(HR/BSL\_HR))$$
$$CO = HR \cdot SV$$
$$MAP = CO \cdot TPR$$
$$FB = FB0 \cdot \left( \frac{IBSL\_MAP}{TVBSL\_MAP\_SHR} \right)^{FB0\_MAP}.$$

In these equations, $SV^{*}$ represents the SV influenced by the negative feedback of MAP, $K_{in\_HR}$, $K_{in\_SV}$ and $K_{in\_TPR}$ represents the zero-order production rate constants and $k_{out\_HR}$, $k_{out\_SV}$ and $k_{out\_TPR}$ represent the first-order dissipation rate constants of HR, SV and TPR, respectively. In addition, *FB0_MAP*, *IBSL_ MAP and TVBSL_MAP_SHR* represent the exponent of the power relationship, the individual baseline values of MAP and typical value of *BSL_MAP* in SHR, respectively. The basic expectations in pharmacodynamics are that physiological turnover rate constants of most general structures and functions should be predictable among species based on allometric principles (Mager *et al.*, 2009; West *et al.*, 1999). Therefore, to extrapolate the extended CVS model to the human situation allometric scaling principles were applied to scale the first order rate constants $k_{out\_HR}$, $k_{out\_SV}$ and $k_{out\_TPR}$ for humans. As allometric models often use an exponent value of 0.75 for clearance and 1 for volume of distribution, the corresponding first order rate constants will have an allometric exponent of -0.25 (Equation 2; Stevens *et al.*, 2012).

$$k_{out\_human} = k_{out\_rat} \cdot \left( \frac{BW_{human}}{BW_{rat}} \right)^{-0.25} \quad (2)$$

In this equation, $k_{out\_human}$ and $k_{out\_rat}$ represent the first-order dissipation rate constant of HR, SV or TPR in humans and rats, respectively. The principles of mass-balance define the zero-order rate constants (Equation 3).

$$K_{in\_HR} = \frac{k_{out\_HR} \cdot BSL\_HR}{1 - FB \cdot BSL\_MAP}$$

$$K_{in\_SV} = \frac{k_{out\_SV} \cdot BSL\_SV}{1 - FB \cdot BSL\_MAP} \quad (3)$$

$$K_{in\_TPR} = \frac{k_{out\_TPR} \cdot BSL\_TPR}{1 - FB \cdot BSL\_MAP}$$

In this equation, *BSL_MAP*, *BSL_HR*, *BSL_SV* and *BSL_TPR* represent the baseline values of MAP, HR, SV and TPR, respectively. This means that the zero-order human production rate constants are intrinsically scaled. Furthermore, Schmidt-Nielsen described how the baseline values of MAP, HR and CO (*BSL_CO*) should be scaled, i.e *BSL_MAP*, *BSL_HR* and *BSL_CO* should be scaled with an allometric exponent of 0, -0.25 and 0.75, respectively (Schmit-Nielsen, 1995). Since MAP=CO*TPR and CO=HR*SV the allometric exponents for scaling *BSL_SV* and *BSL_TPR* follow directly from the allometric exponents for *BSL_MAP*, *BSL_HR* and *BSL_CO* and are 1 and -0.75, respectively. Concerning the extrapolation of the feedback parameter, *FB* was found to be dependent on *BSL_MAP*, which is this same in all species. Therefore, *FB* was assumed to be the same in all species too.

To evaluate the hypothesis that the extended CVS model can be extrapolated to the human situation by applying allometric scaling principles to scale the first order rate constants $k_{out\_HR}$, $k_{out\_SV}$ and $k_{out\_TPR}$ from rats to humans, ideally, the experiments, which were performed to characterize the extended CVS model in rats should be repeated in humans, i.e. the effects of eight drugs with diverse MoA's (amiloride, amlodipine, atropine, enalapril, fasudil, hydrochlorothiazide, prazosin and propranolol) on MAP, HR and CO should be measured in humans. However, performing such a study in humans is outside the scope of this research. As for none of these compounds data on the time course of the effect on all three parameters, i.e. MAP, HR and CO, were published a literature search was

performed to obtain data on the time course of the effect of MAP, HR and/or CO following the administration of cardiovascular drugs. Francheteau *et al.* published data on the effect of nicardipine on MAP and CO following two types of nicardipine infusion (Francheteau *et al.*, 1993); data were digitized using TechDig® version 2.0). Although no HR data was published on the time course of the effect of nicardipine on HR, these data were selected to evaluate if the extended CVS model can be extrapolated to human.

To scale the extended CVS model from rat to human the following assumptions were made (Scenario 1_CVS_model).

i. The dissipation rates can be allometrically scaled with an allometric exponent of -0.25. The body weights of rats and humans were assumed to be 0.3 and 72 kg, respectively.
ii. In rats, feedback (*FB)* was found to decrease with the baseline MAP according to Equation 1. It was assumed that this is equation is applicable in humans too.
iii. The observed baselines can be used. These were slightly different from the allometrically scaled baselines, which may be due to inter-individual variability.
iv. The drug specific parameters can be fixed to values from Francheteau *et al.* (Francheteau *et al.*, 1993; Table 1).

Following these assumptions the extent of the effects of nicardipine on CO and MAP were under-predicted (Figure 6, dashed lines). This could indicate that the feedback is slightly lower in man as compared to rats. In addition, the time course of the effects on MAP and CO was not captured as the observed time lag was much shorter than the predicted time lag indicating that men respond faster than rats. Several hypotheses can be postulated as to why the time course and the extent of the cardiovascular effects are not predicted adequately. First of all, the dissipation rates of HR, SV and TPR do not represent actual degradation rates as for proteins and enzymes. Instead they represent parameters for the delay in response at an organ level and represent several lumped processes as partly reflected by the Guyton model (Guyton *et al.*, 1972). Another hypothesis is that the differences between rats and humans may not be related solely to differences in size. The most obvious difference between rats and humans is that rats crawl, whereas men walk. This could influence both the delay in response and the feedback. To evaluate this last hypothesis, the extended CVS model was extrapolated from rats to humans according to the following assumptions (Scenario 2_CVS_model).

i. The dissipation rates should be multiplied with an unknown factor (this factor was estimated).
ii. In rats, *FB* was found to decrease with the baseline MAP according to Equation 1. It was assumed that this is equation is applicable in humans.
iii. The observed baselines can be used. These were slightly different from the allometrically scaled baselines, which may be due to inter-individual variability.

**Table 1:** Parameters values from the extended CVS model

*Scenario 1*_CVS_model*: dissipation rates are allometrically scaled with an allometric exponent of -0.25*

*Scenario 2*_CVS_model*: dissipation rates are multiplied with an estimated factor of 23.4*

*Scenario 3*_CVS_model*: dissipation rates are multiplied with an estimated factor of 46.7 and FB is estimated*

| Parameter | rat | human | | |
|---|---|---|---|---|
| | | ***Scenario 1*** | ***Scenario 2*** | ***Scenario 3*** |
| ***System-specific parameters*** | | | | |
| $k_{out\_HR}$ (1/h) | 11.6 | 2.95<br>(11.6*(72/0.3)^(-0.25)) | 271<br>(11.6*23.4) | 309<br>(11.6*46.7) |
| $k_{out\_SV}$ (1/h) | 0.126 | 0.032<br>(0.126*(72/0.3)^(-0.25)) | 2.95<br>(0.126*23.4) | 2.36<br>(11.6*46.7) |
| $k_{out\_TPR}$ (1/h) | 3.58 | 0.91<br>(3.58*(72/0.3)^(-0.25)) | 83.7<br>(11.6*23.4) | 95.6<br>(11.6*46.7) |
| FB (1/mmHg) | 0.00664* | 0.00664* | 0.00664* | 0.00817 |
| ***Drug-specific parameters (nicardipine)*** | | | | |
| $E_{max}$ | 0.27** | 0.27** | 0.27** | 0.27 |
| $EC_{50}$ (ng/mL) | 29.1 | 29.1 | 29.1 | 29.1 |
| hill coëfficiënt | 4.52 | 4.52 | 4.52 | 4.52 |

* FB =0.00290*(102/155)^(-1.98)=0.00664

** Emax=(TPReq-TPRmin)/TPReq; TPReq=75/6.08=12.33 mmHg/(L/min); TPRmin=8.96 mmHg/(L/min); Emax=0.27

iv. The drug specific parameters can be fixed to values from Francheteau *et al.* (Francheteau *et al.*, 1993; Table 1).

Following these assumptions the time course and extent of the effect of nicardipine on CO was described adequately, whereas the time course and extent of the effect of nicardipine on MAP were not captured (Figure 6, dotted lines). The multiplication factor was estimated to be 23.4 (Table 1). Although MAP is assumed to be a non-scalable parameter, albeit in the same order of magnitude, the observed *BSL_MAP* in humans (75 mmHg) is actually lower than in rats (102 mmHg). This means that, when applying Equation 1 to calculate *FB*, an extrapolation was made beyond the observed *BSL_MAP* range. Therefore, the second assumption to extrapolate the extended CVS model from rats to humans was changed (Scenario 3_CVS_model).

i. The dissipation rates should be multiplied with an unknown factor (this factor was estimated).
ii. In rats, *FB* was found to decrease with the baseline MAP according to Equation 1. It was assumed that this is equation is not valid in subjects with a *BSL_MAP* lower than 102 mmHg (*BSL_MAP* in normotensive rats). To test this hypothesis *FB* was estimated.
iii. The observed baselines can be used. These were slightly different from the allometrically scaled baselines, which may be due to inter-individual variability.
iv. The drug specific parameters can be fixed to values from Francheteau *et al.* (Francheteau *et al.*, 1993; Table 1).

Following these assumptions the time course and extent of the effect of nicardipine on CO and MAP were described adequately (Figure 6, continuous lines). The estimated *FB* (0.00817 [CI: 0.00740-0.00894] 1/mmHg) (Table 1) was slightly lower than the calculated *FB* from Equation 1 (0.0122 [CI: 0.0108-0.0136] 1/mmHg). Notably, the estimated *FB* does not differ significantly from the value from normotensive rats (0.00664 [CI: 0.00586-0.00742] 1/mmHg). This indicates that it is likely that *FB* does depend on *BSL_MAP*, but not according to a hyperbolic function as *FB* may reach a maximum. In addition, the multiplication factor was 46.7 indicating that the response in humans is very fast, i.e. the half-life's of the effect on HR, SV and TPR are 0.00128, 0.118 and 0.00415 h, respectively.

In conclusion, the extended CVS model can be extrapolated from rat to human, but the extrapolation is not solely depended on size and other factors, such as the fact that men are standing whereas rats are crawling, seem to play an important role. By multiplying the dissipation rates with a certain factor (a high value which results in a very short delay in response in humans), and estimating the feedback, the effect of nicardipine on MAP and CO could be described adequately. As the extrapolation of the extended CVS model was based on data from one compound (nicardipine) it is not certain if the obtained system parameters are drug-independent. Therefore, further research is required to obtain a truly system specific CVS model in humans. Ideally, a study should be performed were the

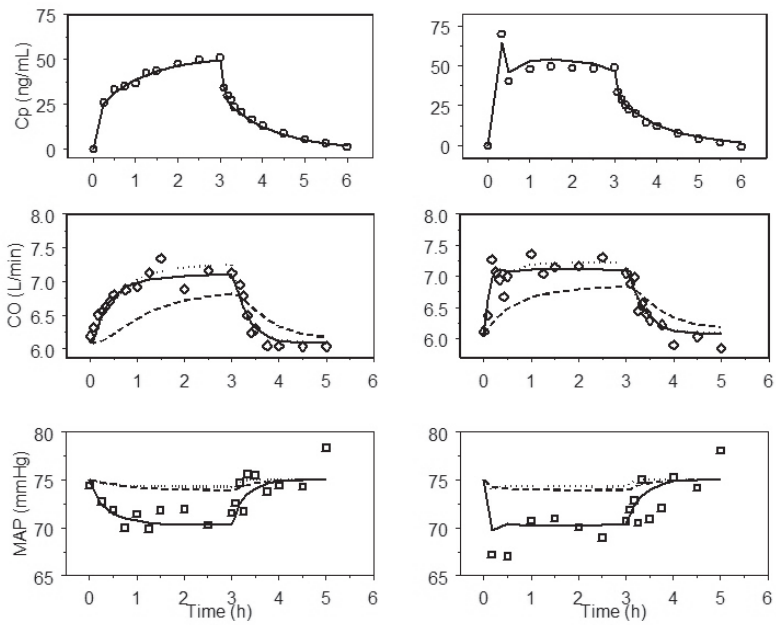


**Figure 6:** Observed (Francheteau *et al.*, 1993) and predicted curves of the extended CVS model to mean plasma concentrations of nicardipine (○), cardiac output data (◊) and mean arterial pressure data (□) following two types of nicardipine infusion.

*Left panel: constant rate infusion; Right panel: variable rate infusion*

*Scenario 1*_**CVS_model**: *Dissipation rates were allometrically scaled with an allometric exponent of -0.25 (dashed lines)*

*Scenario 2*_**CVS_model**: *Dissipation rates are multiplied with an estimated factor of 23.4 (dotted lines)*

*Scenario 3*_**CVS_model**: *Dissipation rates are multiplied with an estimated factor of 46.7 and FB is estimated (continuous lines)*



time course of the effect of different cardiovascular compounds with different MoA's on MAP, HR and CO is measured.

## *Translation of the class/drug-specific target binding and activation model for S1P agonists*

In a next step, it was investigated if the human CVS model, with the parameter estimates fixed, can be applied to the characterization and prediction of the cardiovascular effects of S1P agonists in humans (data on file). To this end, it is discussed how the rat target binding and activation model for fingolimod-P and siponimod may be scaled to man (Supplement).

## General summary, discussion and applications

The objectives of the investigations described in this thesis were 1) to establish a systems pharmacology model to characterize the effects of drugs with different MoA's on the interrelationship between MAP, CO, HR, SV and TPR in a quantitative manner and 2) to apply the model to the quantification of the cardiovascular effects of the S1P receptor modulator fingolimod. A systems pharmacology modeling approach was applied, which allows predicting hemodynamic changes in man on the basis of information from pre-clinical studies. In this section, the development and translation of the system-specific CVS model is summarized and discussed first. Thereafter, the development of the target binding and activation model for S1P agonists is discussed. Finally, a strategy is presented to obtain a quantitative understanding of the *in vitro* to rat to human translation of the cardiovascular effects of S1P agonists.

First, a system-specific CVS model was developed to characterize drug effects on the interrelationship between MAP, CO, HR, SV and TPR in rats. This model can be applied to quantify the hemodynamics of novel cardiovascular compounds. Moreover, it can be applied to elucidate the site of action of novel compounds. In a first step towards the development of a translational system-specific CVS model, the parameters of the rat model were scaled to humans following an unconventional (not based on the allometric scaling principles), but physiologically plausible, scaling approach. The fact that parameters of the rat CVS model were not scaled according to the allometric scaling principles does not imply that cardiovascular effects cannot be scaled from rat to human. Instead, this implies that differences in cardiovascular responses between rats and humans do not solely depended on size, but other factors are also of importance, such as the fact that humans are standing whereas rats are crawling and this should be accounted for. As the translation of the CVS model was based on data from one compound (nicardipine) it is not certain if the obtained system parameters are drug-independent. Therefore, further research is required to obtain a truly system specific and drug independent CVS model in man. Once the human CVS model has been validated, it can be applied to the characterization and prediction of cardiovascular effects of novel compounds in humans.

In a next step, the rat CVS model, with the system-specific parameter estimates fixed, was integrated with a target binding and activation model to quantify the cardiovascular effects S1P agonists in rats, using fingolimod-P as a paradigm compound. This model provided a quantitative understanding of the mechanisms underlying the cardiovascular effects of fingolimod-P. In addition, the effect of siponimod on the CVS was predicted adequately by multiplying the estimated *in vivo* dissociation constants of fingolimod-P for binding to the S1P receptors with the ratio of the potencies of fingolimod-P and siponimod derived from

*in vitro* binding assays. Therefore, it is anticipated that the developed model can be applied to predict the effect of other S1P receptor agonists on the CVS in rats. Subsequently, hypotheses were generated on the rat to human translation of the cardiovascular effects of S1P agonists. Overall, the human CVS model, integrated with the target binding and activation model for S1P agonists from rat, was successfully applied to characterize the magnitude and dynamics of the effect of fingolimod-P and siponimod on HR (data on file).

However, this required several assumptions on the parameter values (Supplement).
To date, no 'gold' standard approach exists for the inter-species translation of cardiovascular effects. In practice, the selected translational approach is often driven by the available data. Here, an approach is presented to obtain a mechanistic and quantitative understanding of the *in vitro* to rat to human translation of the cardiovascular effects of S1P agonists (Figure 7). First, a relationship between *in vitro* binding constants derived from saturation binding experiments using the rat S1P receptor and the apparent *in vivo* rat dissociation constants should be established. Possible differences between these parameters may result from processes that occur solely *in vivo* such as signal transduction. For fingolimod, it was demonstrated that valuable information on the mechanisms underlying its cardiovascular effects can be derived from rat experiments. More precisely, the model structure to characterize the interrelationship between the cardiovascular effects of S1P agonists can be derived from rat experiments and applied to the prediction of



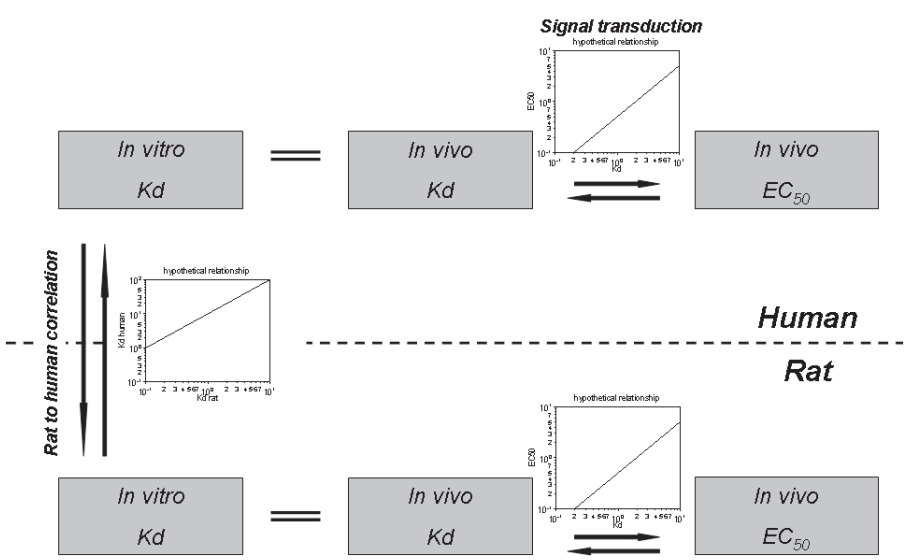


**Figure 7:** Schematic map for the translation of cardiovascular effects of S1P agonists.

cardiovascular effects in humans (Supplement), indicating that the physiological processes are qualitatively comparable between rats and humans. Subsequently, relationships between the *in vitro* dissociation constant for rats and humans and between the *in vitro* and apparent *in vivo* dissociation constants in humans should be established. This requires information on 1) the effects of multiple S1P receptor agonists on MAP, HR and CO in rats and humans and on 2) the corresponding dissociation constants for binding to the rat and human S1P receptors. When these relationships are established, it is foreseen that the magnitude and hemodynamics of the cardiovascular effects of novel S1P agonists, i.e. S1P agonists that were not included in model development, can be predicted directly using parameters estimates obtained from *in vitro* experiments and no further experiments in rats are required. If the proposed approach results in an adequate translational model for the cardiovascular effects of S1P agonists, this would demonstrate that preclinical experiments are predictive for clinical effects, but a quantitative understanding of the processes in the causal chain between drug administration and the change in response over time, including the pharmacokinetics of a drug, target site distribution and receptor binding kinetics, receptor activation and transduction (Ploeger *et al.*, 2009) in rats and humans is required.

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