Towards predictive cardiovascular safety : a systems pharmacology approach

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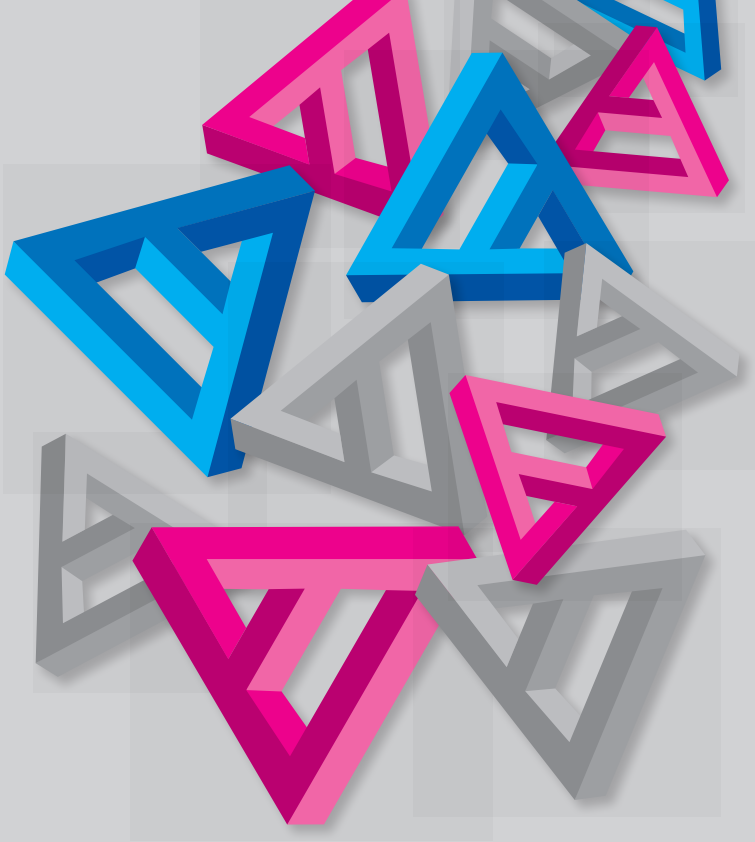

CHAPTER 1

A systems pharmacology approach for predicting drug-induced changes in hemodynamic variables

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). Clinically, hypertension is defined as BP higher than 140/90 mmHg, (i.e. a systolic pressure higher than 140 mmHg and a diastolic pressure higher than 90 mmHg), and affects 44.2 and 27.6 % of the European and American population in the age range of 35 to 64 years, respectively (Wolf-maier *et al.*, 2003). The most common form of hypertension is primary hypertension (also called essential hypertension), of which by definition, the cause is unknown. This complicates the treatment and has led to a "trial and error" treatment strategy based on predefined first-, second- and third-line therapy (Royal College of Physicians, Management of hypertension in adults in primary care. NICE Clinical Guideline 18, 2006). The prevalence of secondary hypertension, i.e. hypertension with an identifiable underlying cause, is much lower. Although in only 5% of the hypertensive patients the cause of hypertension is known, the absolute number of patients affected by secondary hypertension is still high. Secondary hypertension can be caused by various diseases including endocrine and kidney diseases and cancer (Grossman and Messerli, 2012). However, it can also be caused as a side effect of drugs that are prescribed for non-cardiovascular indications, (Sager *et al.*, 2013). This is still an unappreciated cause of secondary hypertension even though a myriad of drugs have been reported to induce a transient or sustained increase in BP, including non-steroidal anti-inflammatory drugs and analgesics, anti-anginogenic therapies that inhibit vascular endothelial growth factor signaling, antidepressant agents, steroids and sex hormones (Grossman and Messerli, 2012). For these specific drug classes the mechanisms of action (MoA) underlying the undesired effects on BP have been elucidated. However, in drug development cardiovascular safety issues occur frequently with novel compounds (Sager *et al.*, 2013). The MoA underlying these undesired BP effects is often not fully understood. This is a major drawback since a quantitative understanding of the pharmacological effects of (novel) drugs on BP control is pivotal from a drug safety point of view. In addition, although clinically hypertension is defined by a clear cut-off value, i.e. BP higher than 140/90 mmHg, it should be noted that the risk of cardiovascular events continuously increases with increased BP levels. Even changes in BP as small as 3 mmHg can have a relatively large influence in certain patient populations (Sager *et al.*, 2013; EMEA, 2004). This underscores the importance of detecting and understanding undesired BP effects of novel compounds.

This thesis focuses on identification of the MoA of drugs with an undesired effect on BP. Moreover, it describes how the magnitude and dynamics of drug effects on the cardiovascular system (CVS) in man can be predicted from pre-clinical investigations, which is important as this determines the benefit-risk ratio of novel drugs. In this chapter, the physiology of the CVS is described first. Thereafter, it is discussed how the parameters of

the CVS can be monitored. Subsequently, the current status with regard to the assessment of drug-induced changes in BP during drug development is reviewed. Finally, the use of systems pharmacology modeling to provide a quantitative understanding of the pharmacological effects of (novel) drugs on the CVS to improve the prediction of the magnitude of the hemodynamic effects in humans is discussed. Although this chapter focuses on undesired effects of drug on BP it should be realized that many of the principles that are discussed are also relevant for drugs with desired effects on BP. However, this is beyond the scope of this chapter.

Physiology of the CVS

The primary function of the CVS, which consists of the heart, blood, and blood vessels and includes the pulmonary and systemic circulation, is the rapid convective transport of oxygen, glucose, amino acids, fatty acids, vitamins and water to the tissues and the rapid washout of metabolic waste products such as carbon dioxide, urea and creatinine (Levick, 2003).

Hemodynamics

The blood flow through the systemic circulation (hemodynamics) is governed by physical laws. Under steady flow conditions, the flow is proportional to the pressure difference between the inlet and outlet pressure (Equation 1) (Levick, 2003).

$$\dot{Q} = K \cdot (P_1 - P_2) \tag{1}$$

In this equation, Q represents the flow, K represents the hydraulic conductance, and P_1 and P_2 represent the inlet and outlet pressure, respectively. As resistance (R) is the reverse of conductance, i.e. 1/K, the basic law of flow can be re-written into Darcy's law of flow, which is similar to Ohm's law for fluid flow (Equation 2).

$$\dot{Q} = \frac{P_1 - P_2}{R} \tag{2}$$

It indicates that resistance equals the difference in pressure needed to drive one unit of flow in steady state, i.e. mmHg per mL/min. In the CVS, flow through the entire systemic circulation equals the cardiac output (CO). The pressure difference is mean arterial pressure (MAP) minus central venous pressure (CVP) and resistance is called total peripheral

resistance (TPR). Therefore, when applying Darcy's law to the CVS, equation 2 translates into Equation 3.

$$CO = \frac{MAP - CVP}{TPR} \tag{3}$$

Since CVP is much smaller than MAP, this equation can be simplified (Equation 4).

$$CO = \frac{MAP}{TPR} \tag{4}$$

In addition, CO equals the volume of blood ejected by one ventricle per unit of time. It is the product of stroke volume (SV) and heart rate (HR) (Equation 5).

$$CO = HR \cdot SV \tag{5}$$

Figure 1: Equations to characterize the hemodynamics of the CVS

In conclusion, the hemodynamics of the CVS are characterized by two equations (Figure 1).

It should be noted that arterial pressure is pulsatile, because the heart ejects blood intermittently. Between successive ejections the systemic arterial pressure decays from a peak of ~120 mmHg to a trough of ~80 mmHg. The pulsatile character of arterial pressure is not captured by these equations. However, this is deemed irrelevant as this thesis focuses on drug effects on MAP.

Blood pressure regulation

The mechanisms of BP regulation by the CVS have been carefully characterized, and the homeostatic principles of the CVS are thoroughly understood. Briefly, MAP is maintained within narrow limits by various regulatory feedback systems which control BP on different time scales (Figure 2). The baroreceptor reflex system is primarily responsible for short term BP regulation at the time scale of seconds. Other systems that regulate BP within seconds include the chemoreceptor reflex and the ischemic response. In addition, several hormonal systems including the renin-angiotensin-aldosterone system (RAAS) (indicated by "Capillary" in Figure 2), and some minor systems, control blood pressure within at

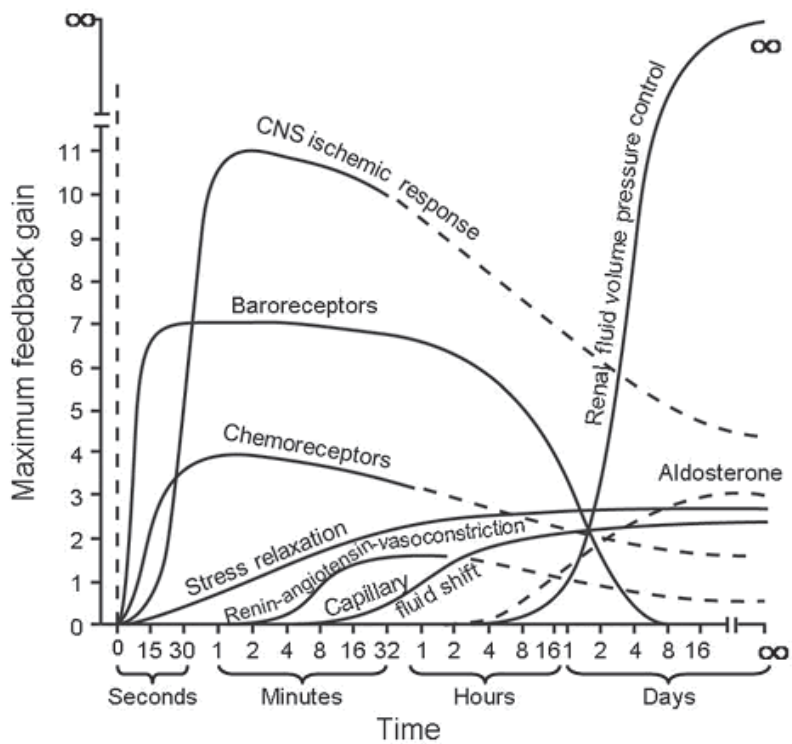

Figure 2: Blood pressure control. Degree of activation, expressed in terms of feedback gain at optimal pressure, of different pressure control mechanisms after a sudden change in arterial pressure. CNS, central nervous system (Okumura and Cheng, 2012)

a time scale of minutes. Finally, the kidney-fluid volume system is responsible for long term BP regulation and affects blood pressure within hours or days (Okumura and Cheng,

2012). In this chapter, first, the baroreflex system and, subsequently, the RAAS will be discussed in more detail.

The baroreceptor reflex system regulates HR and TPR and, thereby, MAP through the sympathetic and parasympathetic nervous system. Baroreceptors are stretch-sensitive mechanoreceptors, which are present in the vena cavae, carotid sinuses and aortic arch. When MAP rises, the carotid and aortic sinuses are distended resulting in stretch and, subsequently, activation of the baroreceptors. Active baroreceptors fire action potentials more frequently than inactive baroreceptors. The greater the stretch the more rapidly baroreceptors fire action potentials. These action potentials are relayed to the brainstem. Baroreceptor activation results in inhibition of the sympathetic nervous system and activation of the parasympathetic nervous system. The sympathetic and parasympathetic branches of the autonomic nervous system have opposing effects on MAP. Sympathetic activation leads to an elevation of TPR and CO via increased contractility of the heart and increased heart rate and, thus, to increased MAP. Conversely, parasympathetic activation leads to decreased CO via a decrease in HR and, thus, to decreased MAP. By coupling sympathetic inhibition and parasympathetic activation, the baroreflex maximizes MAP reduction (Levick, 2003). In a similar manner, sympathetic activation with parasympathetic inhibition allows the baroreflex to elevate MAP.

The RAAS regulates blood volume. If blood volume increases the venous return of blood to the heart increases, resulting in increased SV, CO and MAP. The blood volume is regulated through changes in MAP. Briefly, a decrease in MAP promotes the release of the hormone renin from the kidney into the blood. Renin promotes the production of angiotensin I from angiotensinogen. Subsequently, angiotensin I is converted into angiotensin II by angiotensin converting enzyme (ACE). Angiotensin II constricts blood vessels and promotes renal salt and water retention by direct intrarenal actions and by stimulating and by stimulating the release of aldosterone (Cleophas, 1998). Aldosterone acts on the distal tubules and collecting ducts of the nephron, increasing reabsorption of ions and water in the kidney. This causes the conservation of sodium, secretion of potassium, increase in water retention, and increase in MAP.

Drug effects on the cardiovascular system

The cardiovascular system can be influenced by drugs through a variety of different, and often complex, mechanisms. However, basically, most compounds directly influence HR, SV and/or TPR as elucidated for a selection of commonly applied cardiovascular drugs in Table 1. Due to the different feedback mechanisms that regulate the CVS the direct effect

Table 1: A selection of commonly applied cardiovascular drugs and their mechanism of action (this thesis).

Compound	Class	Mechanism of action	Effect
amiloride	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).	SV
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 *et al.*, 1997; Perez-Reyes *et al.*, 2009).	TPR
atropine	M2 receptor antagonist	Muscarinic (M2) receptor antagonist (MRA) is an agent that blocks the activity of the muscarinic acetylcholine receptor. It causes tachycardia by blocking vagal effects on the sinoatrial node. Acetylcholine hyperpolarizes the sinoatrial node which is overcome by MRA and thus increases the heart rate	HR
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). In addition, aldosterone acts on the distal tubules and collecting ducts of the nephron, the functional unit of the kidney. Decreased levels of aldosterone, cause the depletion of sodium, conservation of potassium, decreased water retention, and decreased blood pressure	TPR and SV
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
HCTZ	diuretic	See amiloride	SV
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 *et al*., 2008*)*.	HR

of compounds are translated into differential effects on the other variables of the CVS, i.e. MAP, CO, HR, SV and TPR (this thesis). For example, fasudil is a calcium channel blocker, which decreases TPR through smooth muscle cell contraction (direct effect). Since MAP equals the product of TPR and CO, MAP is also decreased. As a result of the different

feedback mechanisms regulating the CVS HR, SV and CO are increased after administration of fasudil (indirect effect).

Monitoring the variables of the cardiovascular system

Detection of drug-induced changes in the hemodynamics may be influenced by the frequency and type of cardiovascular measurements during a study (Sager *et al.*, 2013). As mentioned in the section "Physiology", the hemodynamics of the CVS are characterized by five basic variables, i.e. MAP, HR, CO, SV and TPR. In experimental and clinical pharmacology measuring MAP and HR is common practice. However, measuring CO, SV and TPR is not due to a lack of a perfect 'gold' standard measuring technique as detailed further in this section. Moreover, most measurement techniques require invasive instrumentation procedures, which limits the applicability of these techniques. Nevertheless measuring CO is important, because when MAP, HR and CO are measured SV and TPR can be derived using Equations 4 and 5. This provides a full understanding of drug effects on all variables of the CVS instead of on only two, i.e. MAP and HR. Moreover, since drug effects on CO and TPR may be much larger than anticipated from the observed responses on MAP and HR, measuring CO provides powerful information to detect patho-physiological conditions. In this section, it is first discussed how MAP and HR can be measured in conscious animals and in humans. Subsequently, it is discussed how CO can be measured.

Despite the fact that MAP is one of the most commonly measured hemodynamic parameters throughout drug development, there is no uniformly agreed methodology for how MAP should be measured (Sager *et al.*, 2013). Typically, in preclinical research, dedicated telemetry studies are performed to evaluate acute effects of drugs in conscious rats, dogs or nonhuman primates. In these studies, MAP and HR are usually continuously recorded using indwelling catheters (Sager *et al.*, 2013). Since MAP and HR are continuously recorded over several days this provides information for detecting 1) the diurnal profile, 2) direct and delayed drug effects and 3) short and long term effects on MAP and HR. In addition, another noninvasive technique to measure MAP is available, i.e. oscillometric tail cuff with jackets, but this technique requires further refinement to improve system sensitivity to detect smaller changes in MAP (Ward *et al.*, 2012, Sager *et al.*, 2013). In human, MAP is measured noninvasively using manual or digital sphygmomanometers (blood pressure meters) or by ambulatory blood pressure monitoring (ABPM) and HR can be measured by ABPM, electrocardiograph (ECG) or pulse oximeters. The information obtained on changes in MAP and HR by ABPM is comparable to the information obtained from telemetry studies in conscious animal. Therefore, ABPM measurements are uniquely suited to detect the dynamics of drug effects on MAP and HR. In addition, the variability in measurements is much smaller with ABPM as compared to measurements from sphyg-

momanometers. Especially when MAP and HR are measured in the clinic the variation in MAP and HR measurements can be very large, e.g. because of the white-coat effect (i.e. a transient elevation in MAP that does not appear to be linked to target organ damage or prognosis, but to the anxiety or stress that can be experienced during a visit to a physician). This should be taken into account when assessing drug-induced changes in the CVS. Although measuring CO could provide a better understanding of underlying pathophysiological processes, this has not been integrated into daily practice due to difficulties associated with invasive instrumentation procedures in both animal and human (Vincent *et al.*, 2011; Doursout *et al.*, 2001). In conscious and freely moving rats, CO can be measured with a variety of techniques (Doursout *et al.*, 2001), including the Fick method, thermodilution, microsphere detection, impedance cardiography, transit ultrasound and electromagnetic flowmetry (Tsuchiya *et al.*, 1978; Gotshall *et al.*, 1987). Only the last method allows immediate observation of phasic aortic flow patterns and has been used to estimate cardiac function indirectly by means of derivatives of phasic aortic signals (deWildt and Sangster, 1983). Another method of interest for measurement of blood flow is the use of pulsed Doppler flow probes. This method is based on the direct relationship between blood velocity and volume flow. This method of measuring CO has not been used in many species. However, it has been claimed that these measurements are accurate in rats (Gardiner *et al.*, 1990). In human, the pulmonary artery catheter, also called Swan-Ganzkatheter, has long been considered optimal for hemodynamic monitoring, allowing for the almost continuous, simultaneous recording of pulmonary artery and cardiac filling pressures, cardiac output and oxygen saturation. However, the technique is invasive. Moreover, there is increasing evidence that this method is neither accurate nor effective in guiding therapy (Vincent *et al.*, 2011). There are many different monitoring systems available ranging from the highly invasive pulmonary artery catheter to the completely non-invasive bioimpedance/bioreactance, CO_2 rebreathing and echocardiography and echo-Doppler techniques. In general, variability in CO measurements is large. Classifying them according to how accurate or precise they are is difficult, in part because of the lack of a perfect 'gold' standard for comparison (Vincent *et al.*, 2011). Most devices have been evaluated by comparing their results with those obtained by intermittent thermodilution from the pulmonary artery catheter as the reference, although this technique has its own limitations and may not represent the gold standard best. The bioimpedance/bioreactance technique has been used for physiological studies in healthy individuals (Marque *et al.*, 2009). This technique has the advantage that it allows continuous recording of CO. However, further investigation is required to investigate if this technique is reliable in critically ill patients (Vincent *et al.*, 2011).

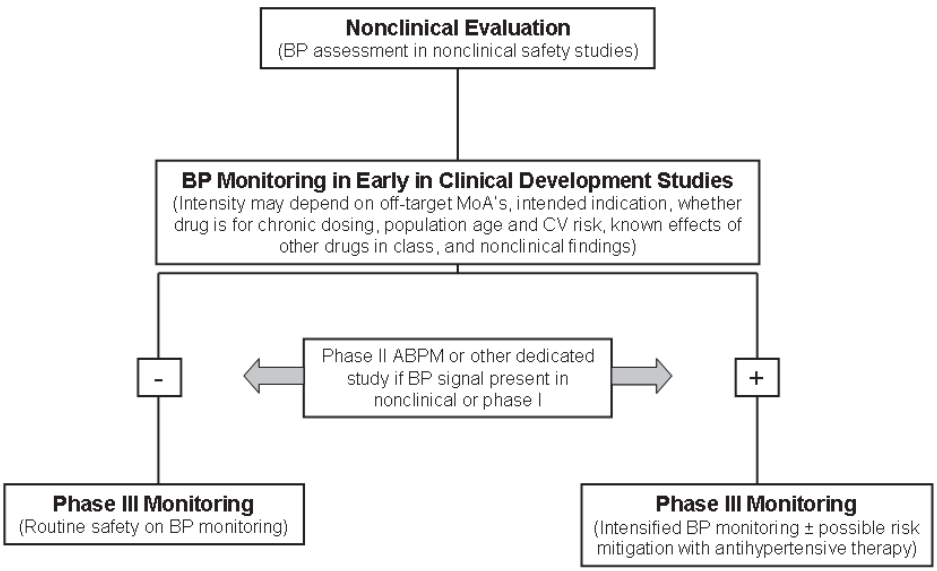

Figure 3: MAP Assessment Development process (Sager *et al.*, 2013)

Assessment of drug effects on the CVS in drug-development

In general, drug effects on MAP are assessed in all phases of the drug-development process (Figure 3). In preclinical development, safety studies are performed ranging from *in vitro* assays to fully integrated *in vivo* animal models (Sager *et al.*, 2013). The translation of these effects from preclinical to clinical development is often not fully understood and it is under debate whether preclinical studies are predictive for clinical studies. A recent meta-analysis comparing the effects of small molecules on diastolic BP measured in conscious dog telemetry studies and the single-ascending dose phase of first-in-human studies suggest that a 5% change in diastolic BP in dog telemetry studies would provide for 37% sensitivity (probability of dog correctly identifying a positive phase I outcome) and 60% specificity (probability of correctly identifying negative phase I outcome) (Sager *et al.*, 2013). As the physiology of the CVS is comparable between species (Schmidt-Nielsen, 1995) it is plausible that drug effects on the CVS are comparable between species too, albeit that there may be quantitative differences resulting from differences in size and function. Therefore, in cases where at first site the drug effect observed in animals seems to be not predictive for human, this may be explained by an incomprehensive understanding of the translation (the system differences). Another explanation may be that the interpretation of the results is not adequate, e.g. because of the lack of uniformity in the nonclinical approaches and the variability in the MAP measurements in clinical development (section “Monitoring the parameters of the CVS”). Therefore, an integrative

approach to data interpretation would appear most desirable (section "Modelling the CVS").

Although undesired cardiovascular drug effects are usually detected in preclinical studies, the clinical relevance of these effects often only becomes apparent in the clinical development when drug effects are evaluated in healthy volunteers and/or in the target population. The clinical relevance of drug-induced cardiovascular effects is determined by many factors, such as the benefit-risk profile, treatment indication and duration of treatment and the cardiovascular risk of the target population. The clinical evaluation of drug effects on MAP involves multiple considerations, which are usually based on the presumed MoA underlying the undesired effects on MAP (Figure 3). However, in contrast to the detailed understanding of the physiologic regulation of MAP, the mechanisms underlying the effects on MAP of compounds with a novel MoA are often less clear. This is a major drawback since a quantitative understanding of the pharmacological effects of (novel) drugs on MAP control is pivotal with regard to safety, the prediction of the magnitude of hemodynamic effects in human and the adequate assessment during clinical development. For example, if intensified MAP monitoring in phase III studies is required to investigate possible risk mitigation with antihypertensive therapy it is pivotal to understand the MoA of the compound in order to adequately reverse an adverse effect on MAP (Sager *et al.*, 2013). This underscores the importance of understanding these effects early in preclinical development since this could improve the anticipation of the magnitude of hemodynamic effects in humans.

Modelling the CVS

Pharmacometrics is the scientific discipline that uses mathematical models based on biology, pharmacology, physiology, and disease for *in vivo* quantification of drugs effects. Models in pharmacometrics can be differentiated by their area of application, for example "pharmacokinetic-pharmacodynamic (PKPD) models", "disease models", "trial execution model" or any combination of these (Zhang *et al.*, 2008). In this section, the focus is on PKPD modeling. The primary objective of PKPD modeling is to identify key properties of a drug *in vivo*, which allows the characterization and prediction of the time course of drug effects under physiological and pathological conditions. A pharmacokinetic (PK) model characterizes the time-course of the drug concentration and a pharmacodynamic (PD) model characterizes the relationship between exposure and pharmacological effect. PKPD modeling is applied in all stages of drug development and has proven to be a useful tool to support decision making in the key steps of drug development process (Breimer and Danhof, 1997). Within this context, PKPD modeling constitutes the theoretical basis for

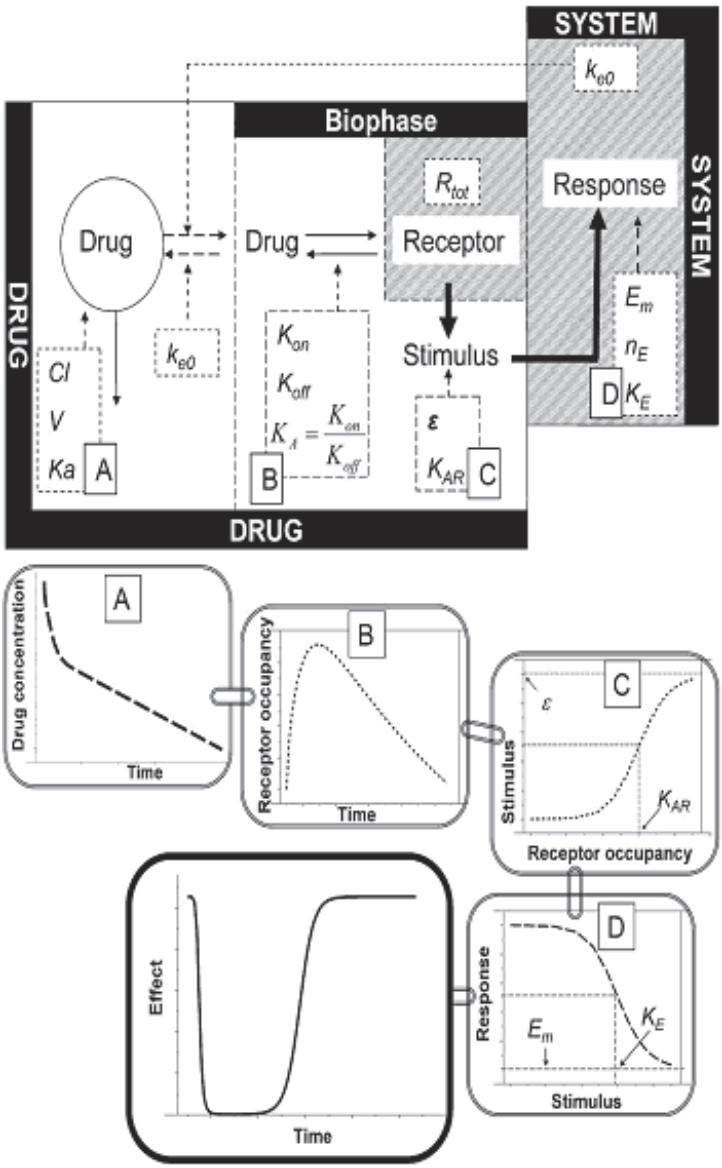

Figure 4: Processes in the causal chain between drug administration and the change in response over time, including the pharmacokinetics of a drug (process A), target site distribution and receptor (target) binding kinetics (process B), receptor activation (process C) and transduction (process D).These processes are characterized by receptor theory models incorporated in mechanism-based PK-PD models (Ploeger *et al.*, 2009).

the selection of drug candidates, lead optimization, and the optimization of early proof-of-concept clinical trials on the basis of information from preclinical studies (Danhof *et al.*, 2007; Danhof *et al.*, 2008). PKPD modeling has developed from an empirical and descriptive approach into a scientific discipline based on the (patho-) physiological mechanisms behind PKPD relationships. As a result PKPD models range from purely empirical models, i.e. descriptive models to mechanism-based and systems pharmacology models with an increasing level of complexity an increasing level of predictive power. Mechanism-based models differ from empirical models in that they quantitatively characterize specific processes in the causal chain between drug administration and effect. A key element of mechanism-based modelling is the explicit distinction between parameters to describe drug-specific properties and biological system-specific properties. Drug-specific parameters (i.e., receptor affinity, intrinsic efficacy) describe the interaction between the drug and the biological system in terms of target affinity and target activation, whereas system-specific parameters describe the functioning of the biological system (Figure 4). The explicit distinction between drug-specific parameters and biological system-specific parameters is crucial to the prediction of *in vivo* drug effects (Danhof *et al.*, 2007; Ploeger *et al.*, 2009). Therefore, mechanism-based PKPD models have much improved properties for extrapolation and prediction as compared to empirical models. Systems pharmacology models attempt to inject biological realism to bring molecular or cellular detail closer to high-level, functional behavior (Vicini and van der Graaf, 2013). Where mechanism-based models focus on pathways, the level of complexity in systems pharmacology is increased further by focusing on networks and the interaction between different components of the network. This can be on different levels in the biological system ranging the organ level to the cellular level. Focusing on networks instead of pathways has the advantage that drug effects on interrelationships between the components of a network, i.e. different pathways, can be characterized and predicted.

Systems biology is an approach to understanding biological processes as integrated systems instead of as isolated parts. The influence of systems biology has often been at a very fundamental (cellular or subcellular) biological scale, difficult to mechanistically link to higher-order tissue or organ systems. The Guyton and Coleman, which describes the physiology of the CVS in great detail model (Guyton *et al.*, 1972), represents an example of a systems biology model (Figure 5). This model is a systems model of the human circulatory physiology, capable of simulating a variety of experimental conditions and contains a number of linked subsystems related to the circulation and its neuroendocrine control. The complete model consists of separate modules, each of which characterizes a separate part of the physiological subsystem. The “Circulation Dynamics” part is the primary system, to which other modules/blocks are connected. The other modules characterize

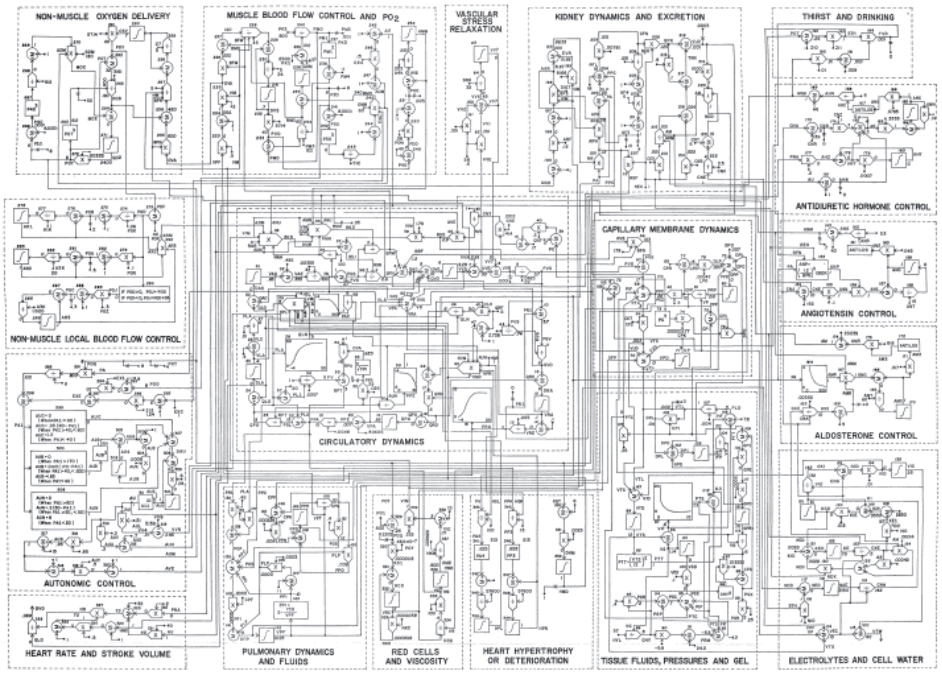

Figure 5: A systems analysis diagram for the full Guyton model describing circulation regulation (CellML, 2008)

the dynamics of the kidney, electrolytes and cellular water, thirst and drinking, hormone regulation, autonomic regulation, cardiovascular system etc., and these feed back on the central circulation model. The Guyton model has provided the scientific basis for the understanding of long-term BP control (Montani and Van Vliet, 2009).

Typically, systems biology is not concerned about therapeutic intervention; rather, deep study of targets and pathways is its focus. In that respect systems biology models differ from PKPD models, which aim to characterize drug effects. Next to this obvious difference, these models also differ in the level of detail included in the model and in the model selection criteria and the criteria for parameter identification. In PKPD modelling a data driven, top-down approach is followed starting at a parsimonious descriptive level and subsequently adding more complexity to better understand the system. These models are developed and selected by finding a middle ground between the model's complexity and its descriptive power. Such middle ground can be based on statistical principles (e.g., balancing number of parameters and goodness of data fitting). The driver is invariably parsimony — in other words, selection of a model whose complexity is "just right" (least complex with the fewest parameters), given the data. On the other hand, systems biology models are inherently complete and fully mechanistic and one follows a bottom-up approach, starting from the level of molecular pathways (Ploeger *et al.*, 2009). In systems

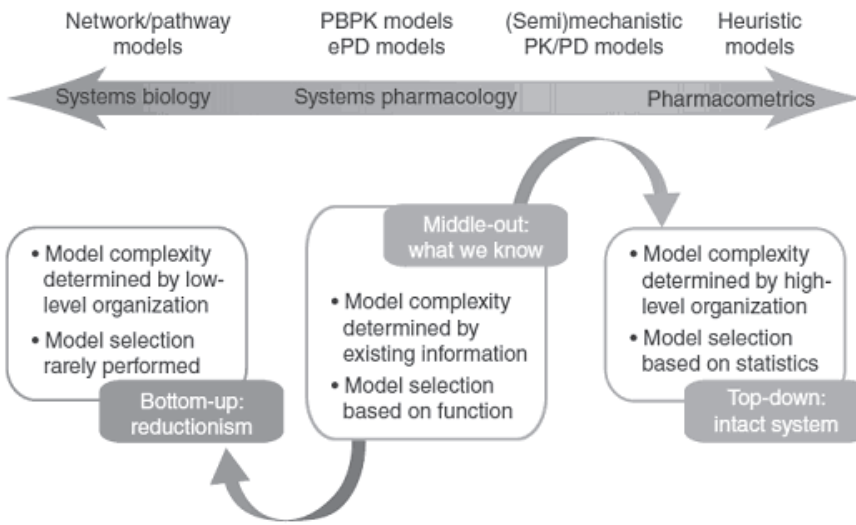

Figure 6: A graphical summary of bottom-up, middle-out, and top-down approaches to model development and their relationship to various model types currently applied in drug discovery and development. In bottom-up approaches, low-level information determines the model, and this often remains conceptual. In top-down approaches, high-level organization and information determine interpretative models. In middle-out approaches, the driving force is available information, and models are selected and built on the basis of functional behavior. In this framework, systems pharmacology can be regarded as an approach to integrate the desirable features of the various model types spanning the spectrum between systems biology and pharmacometrics (Vicini and van der Graaf, 2013).

biology, model selection is rarely performed. Systems pharmacology provides a middle-out approach. As discussed earlier the level of detail in the model is increased further as compared to mechanistic models as networks instead of pathways are characterized in a quantitative manner on different levels in the biological system ranging the organ level to the cellular level. Thereby, the level of detail included in these models middles the level of detail in systems biology models and empirical models. Next to the statistical criteria for model selection, systems pharmacology models are selected based on their function (Figure 6).

Although in many therapeutic areas PKPD modeling has evolved from empirical modelling to mechanistic or systems pharmacology modeling, with examples in diverse areas such as central nervous system disease (Geerts *et al.*, 2013), osteoporosis (Post *et al.*, 2013; Peterson and Riggs, 2012), endometriosis (Riggs *et al.*, 2012) and safety (Lippert *et al.*, 2012), PKPD modeling did not exceed the stage of empirical modeling in the area of cardiovascular disease. For several antihypertensive drugs, no clear relationship be-

tween drug concentration and its effect on MAP has been reported (Gomez *et al.*, 1989; MacGregor *et al.*, 1983; Hansson *et al.*, 1974). This is probably the result of initial studies in which relatively high doses were administered with exposures in the upper part of the sigmoid concentration–response curve, resulting in effects all close to the maximum response (van Rijn-Bikker *et al.*, 2013). Furthermore, the description of the concentration–effect relationship for antihypertensive drugs is often confounded by a failure to collect sufficient pharmacodynamic data, a failure to identify and account for the fact that the MAP-lowering effect develops over a number of weeks, and a failure to account for circadian variability in the diurnal MAP profile (Meredith, 1997). On the other hand, the concentration–effect relationship for angiotensin converting enzyme inhibitors, calcium antagonists and alpha blockers have been successfully established (Bellissant and Giudicelli, 1998; Bellissant and Giudicelli, 2001; Donnellyv, 1989; Donnelly *et al.*, 1988; Donnelly *et al.*, 1993). These models may be classified as empirical models. To date no mechanism-based, mechanistic or systems pharmacology models exist that provide an integrated description of the effects of drugs on the CVS 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. However, 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 distinguished. The fact that no systems pharmacology models are available to characterize drug effects on the CVS is a major drawback since these models are uniquely suited to provide a quantitative understanding of the pharmacological effects of (novel) drugs on the CVS, which is pivotal with regard to drug safety. Moreover, understanding these effects early in preclinical development could improve the anticipation of the magnitude of hemodynamic effects in humans.

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