PK/PD modeling of β -blockers in cardiovascular disease: an update

β -blockers play a central role in the treatment of the various main cardiovascular diseases, including hypertension, coronary artery disease and systolic heart failure. As a therapeutic class, β -blockers form a heterogeneous family that differs in their pharmacokinetic (PK) and pharmacodynamic (PD) properties, highlighting the relevance of the extensive evaluation of PK/PD properties of these agents in order to optimize the outcomes of the treatment of cardiovascular diseases. Preclinical and clinical studies using PK/PD models have been able to identify different factors associated with cardiovascular response of β -blockers, including age, pharmaceutical formulation and genetic polymorphism, among others. PK/PD modeling of β -blockers can also be attractive for the early detection of poor responders and the optimization of dose regimen in their different indications.

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The introduction of β -blockers into clinical practice in the early 1960s has represented a major advance in cardiovascular pharmacotherapy and has clarified the role of the sympathetic nervous system in the etiology of diverse cardiovascular diseases [1]. Nowadays, β -blockers play a central role in the treatment of various main cardiovascular diseases; European guidelines continue to consider β -blockers as first-line therapy of hypertension, stable coronary artery disease, systolic heart failure and ventricular arrhythmias [2–5].

As a therapeutic class, β -blockers form a heterogeneous family that differs in their pharmacokinetic (PK) and pharmacodynamic (PD) properties [6]. Although all β -blockers are able to competitively block the β_1 -adrenoceptor, they differ in the receptor selectivity, partial or inverse agonism, membrane stabilizing effect and presence of additional vasodilatory properties (Table 1) [6,7].

Taking into account differences in the PDs profile, β -blockers are classified as first-generation (nonselective), second-generation (selective blockade of β_1 -adrenergic receptor) and third-generation (β -blockers with vasodilating action) [8]. Vasodilatory effect of carvedilol and nebivolol is related to α -adrenoceptor blockade at the vascular smooth muscle cells and to release in endothelium-derived nitric oxide (NO), respectively [9].

At a PK point of view, β -blockers mainly differ in the degree of gastrointestinal absorption, degree of hepatic first-pass metabolism, lipid solubility, protein binding, brain penetration, concentration within the cardiac tissue, rate of hepatic biotransformation and renal clearance (Table 1) [6,7]. In addition, as β -blockers contain chiral centers and are administered as racemate, the existence of enantioselective PK and PDs properties represents another important feature of this therapeutic class [10,11].

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Table 1. Main pharmacokinetic and pharmacodynamic properties of β -blockers.

	β_1 -selectivity	Intrinsic sympathomimetic activity	Membrane stabilizing activity	Vasodilator effect	Lipophilicity	Elimination
First-generation						
Nadolol	0	0	0	0	Low	Renal
Pindolol	0	++	+	0	High	Renal/hepatic
Propranolol	0	0	++	0	High	Hepatic
Second-generation						
Acebutolol	+	+	+	0	Moderate	Hepatic
Atenolol	++	0	0	0	Low	Renal
Bisoprolol	++	0	0	0	Moderate	Renal/hepatic
Esmolol	++	0	0	0	Low	Renal
Metoprolol	++	0	+	0	High	Hepatic
Third-generation						
Betaxolol	++	0	+	+ (Blockade of calcium channel)	Moderate	Hepatic/renal
Bucindolol	0	+	0	+ (α_1 -blockade)	Moderate	Hepatic
Carteolol	0	+	0	+ (α_1 -blockade)	Low	Hepatic/renal
Carvedilol	0	0	++	++ (α_1 -blockade)	Moderate	Hepatic
Celiprolol	+	+	0	+ (β_2 Agonism)	Moderate	Renal
Labetalol	0	+	+	++ (α_1 -blockade)	Low	Hepatic
Nebivolol	++	0	0	++ (NO availability increases)	Moderate	Hepatic

+: Modest effect; ++: Strong effect; 0: Absence of effect; NO: Nitric oxide.

For instance, only the S-enantiomer of β -blockers binds to β -adrenoceptor, whereas the R-enantiomer is mainly inactive for first- and second-generation β -blockers agents of the therapeutic group [11]. In the case of third-generation congeners, both R- and S-enantiomer of carvedilol show α_1 -adrenergic blocking activity and L-nebivolol induces vasodilatation by an endothelial-dependent mechanism [10].

The great diversity of PK and PD properties of the available β -blockers highlights the relevance of the extensive evaluation of PK and PD properties of these agents in order to optimize the outcomes of the treatment of cardiovascular diseases [7]. In this context, differences in the PK/PD of β -blockers seem to influence in the efficacy and safety of these agents in the clinical setting. Effectiveness of β -blockers in the prevention of stroke is lower than other antihypertensive agents due to a lesser ability to reduce central blood pressure and to attenuate blood pressure variability [2,12]. A recent meta-analysis has shown that β -blockers seem to be inferior to other classes of blood pressure lowering drugs for the prevention of major cardiovascular disease events, stroke, renal failure and all-cause mortality in patients with hypertension [13].

In addition, β -blockers exert deleterious metabolic effects, including insulin resistance, reduced glycemic control and dyslipidemia [14]. Nevertheless, European guidelines recognize differences in the PDs profile of β -blockers that can influence outcomes in the management of cardiovascular diseases [2]. The limitations of traditional nonvasodilating β -blockers are not shared by third-generation agents, such as celiprolol, carvedilol and nebivolol, which are able to reduce central pulse pressure and aortic stiffness [2]. On the other hand, numerous clinical studies have established that third-generation β -blockers are associated with more favorable profile on glucose and lipid profiles than non-vasodilating β -blockers [14].

Taking into account the diversity in PK and PDs properties of β -blockers and its impact in clinical outcomes, a more detailed knowledge of PK/PD properties may improve the selection of the best β -blocker and its dosing regimen in the management of cardiovascular diseases. The aim of the present review was to update the rationale, utility and findings of PK/PD modeling of β -blockers in cardiovascular research. Data for the review article were identified by searches of PubMed and Google Scholar using the following terms: ' β -blockers',

'pharmacokinetic/pharmacodynamic', 'PK/PD' and 'modeling'.

Rationale of PK/PD modeling applied to β -blockers

Knowledge of PK and PD properties of β -blockers can be improved by the design of PK/PD models in both basic and clinical setting in order to estimate relevant parameters for drug therapy [15]. PK/PD models build a bridge between the time course of drug concentrations of β -blockers and the magnitude of the observed pharmacological response [16]. Different mathematical models are used to describe the link between PK and PD of a drug, allowing the estimation of PD parameters, such as effective concentration to yield half-maximal response (EC_{50}) and maximal efficacy (E_{max}) [17].

PK/PD modeling requires the simultaneous assessment of drug tissue concentrations and drug response at multiple time points [18]. Measurements of the active compound should be performed with fully validated analytical methods [17]. Although ideally drug levels at the target site should be measured as PK input of a PK/PD model, in most situations this is not possible and frequent plasma sampling is the only alternative [19]. Drug response represents any change in physiological and biochemical parameters induced by the administration of a drug. Measurement of the effect needs to meet validation parameters, such as continuity, sensitivity, objectivity and repeatability [19].

Different properties of β -blockers justify the development of PK/PD models for the assessment of relevant pharmacological parameters. In first place, β -blockers mainly act by reversible antagonism of β -adrenoceptors inducing diverse cardiovascular effects – blood pressure and heart rate reduction, attenuation of vascular sympathetic activity, among others [7]. Therefore, it is expected that the magnitude of the cardiovascular response induced by β -blockers is strongly related to their tissue concentrations [15]. The main cardiovascular parameters influenced by β -blockers, especially blood pressure and heart rate, meet the validation requirements of measured effects, including continuity, sensitivity, objectivity and repeatability [20]. In addition, blood pressure and heart rate seem to be excellent biomarker of the long-term benefits of β -blockers in hypertension and cardiac failure, respectively [20–22]. Blood pressure has been established as a surrogate end point of the degree of reduction of major cardiovascular events in hypertensive patients, based on the basis of epidemiologic data and numerous clinical trials [20]. In this context, after adjusting for the blood pressure reduction, only small differences were found in the incidence of cardiovascular morbidity and mortality comparative clinical trials of antihypertensive drug

classes [2]. On the other hand, heart rate determines myocardial oxygen demand, coronary blood flow and myocardial performance and plays a central role in the adaptation of cardiac output (CO) to metabolic needs [22]. Increase in heart rate contributes to adverse outcome in the general and in patients with chronic heart failure [22]. Taking into account that heart rate increases to compensate for the deficit of the heart pumping action, it can be considered a good biomarker both in the diagnosis and prognosis of patients with heart insufficiency [22]. Meta-analysis of clinical trials, including almost 20,000 patients, has found that the degree of heart reduction induced by β -blockers is associated with a reduction of cardiovascular outcomes and death [22].

In second place, plasma or tissue levels of β -blockers can be easily monitored by the use of well-validated chromatographic methods [7]. As the majority of β -blockers and their metabolites have natively fluorescence properties, their tissue levels can be continuously assessed by means of highly sensitive analytical methods, especially liquid chromatography (LC) coupled to fluorescence detection [23]. In addition, taking into account the stereoselectivity of the pharmacology of these drugs, several enantioselective chromatographic methods have been developed for the separation and quantification of R- and S-enantiomers of β -blocker [24,25].

PK/PD models for β -blockers

Although measurement of blood pressure lowering effect induced by β -blockers and their tissue concentrations is technically straightforward, it is surprising that accurate information on many concentration–response relationships in hypertension has been accrued only in the 18 years [26,27]. Initial thought that β -blockers show no clear relationship between drug plasma concentrations and their blood pressure lowering effect were based on early studies which used high doses that produced drug concentrations on the upper plateau of the maximal effect limiting the exploration of the lower part of the dose–response curve [27]. Other confounding factors that have contributed to the lack of PK/PD relationship of cardiovascular effects of β -blockers include the lack of individual evaluation of PK/PD parameters, inability to collect PD data from a single patient, failure to identify temporal delay in the onset of the pharmacological response, use of narrow concentration range or doses rather than concentrations [26,27]. More recently, improving knowledge of PK/PD principles has allowed revealing a clear relationship between plasma levels of β -blockers and cardiovascular effects, including blood pressure lowering response and chronotropic action [7,15].

Several methodological aspects need to be considered for the development of an adequate PK/PD model for the estimation of relevant cardiovascular parameters of β -blockers (Table 2). Different PDs models have been developed to describe the relationship between plasma concentration of cardiovascular drugs and the response, including, linear, log-lineal, sigmoidal E_{max} and modified E_{max} model [7]. The selection of an inadequate PK/PD model might lead to erroneous interpretation and inaccurate estimation of PK/PD parameters. For instance, PK/PD studies that evaluate α -blockers and calcium channel blockers use the linear PD model, which has several pitfalls, including the inability to predict maximal response [7]. In addition, accurate estimation of PK/PD parameters using the E_{max} model needs the assessment of the complete PD range after a single dose of the drug. If data are represented by the sigmoidal E_{max} model without attaining a clear maximum, estimation of E_{max} and EC_{50} is extremely variable while their ratio (E_{max}/EC_{50}) is far less so [28]. For some antihypertensive drugs, such as calcium channel blockers, use of large doses is precluded in PK/PD studies due to excessive hypotensive response [29]. An alternative PK/PD model designed by Schoemaker *et al.* [28], which is based on the replacement of EC_{50} by S_0 (initial sensitivity to the drug) in the E_{max} equation, allows a better estimation of PK/PD parameters when maximal response cannot be attained. Nevertheless, experimental studies with β -blockers have shown the possibility to achieve maximal response with large doses without

inducing toxic effects or excessive blood pressure reduction [30–32].

Another aspect that must be considered is the existence of a time delay in the cardiovascular response of β -blockers with regard to their plasma concentrations. In most PK/PD studies that evaluate the effect of β -blockers on heart rate and blood pressure, maximal response is achieved latter than maximal plasma concentration, suggesting the existence of a temporal disconnection between drug exposure and PDs. In this case, plasma concentrations cannot be directly linked to drug effect and more complex PK/PD models are needed [33]. The effect compartment model and the physiological indirect response model represent the most applied PK/PD models in the evaluation of β -blockers (Figure 1) [7]. The effect compartment model considers a hypothetical effect compartment as an additional compartment of a PK compartment model, representing the drug concentration at the effect site [19]. According to this PK/PD model, the delay in the onset of cardiovascular action of β -blocker is due to the time required for distribution of the drug in the biophase. Many mechanisms contribute to the blood pressure lowering effect induced by β -blockers, including reduction in myocardial contractility and CO, diminishment of renin secretion, modification of sympathetic nervous system control at the level of the CNS, a change in baroreceptor sensitivity, an alteration in peripheral adrenergic neuron function and an increase in prostacyclin synthesis [34]. Therefore, delay in the antihypertensive response to β -blockers can be explained by the time spent to achieve relevant concentrations at their different target sites.

A second explanation for the delay in the onset of cardiovascular effects of β -blocker could be the fact that the mechanism of action of these drugs involves inhibition of physiological process involved in the elaboration of the clinical expression of observed effect. For instance, vascular sympathetic tone can be considered as a physiological parameter constantly that is produced through zero-order kinetics (K_{in}) and removed in first-order kinetics with a rate constant K_{out} (Figure 2). In this case, third-generation β -blockers with α -blocking properties, such as carvedilol, are able to inhibit the production of the sympathetic tone (inhibition of K_{in}), thereby affecting its magnitude [31].

As different model are available to account for delay in β -blocker response with regard to plasma levels, it is important to select the most adequate PK/PD model by comparison of goodness of fit parameters. In this context, Liu *et al.* [35] have compared the indirect response and the effect-compartment link model for the estimation of carvedilol effects on blood pressure measured by tail-cuff manometry in 20 male healthy

Table 2. Methodological aspect to consider in pharmacokinetic/ pharmacodynamic modeling of β -blockers.	
Aspect	Comment
Pharmacokinetic data	Total unbound plasma concentrations Plasma microdialysate levels Stereoselective plasma levels Active metabolite plasma levels
Pharmacodynamic end point	Resting heart rate Isoprenaline or exercise induced tachycardia Blood pressure Vascular sympathetic activity
PK/PD models	Direct inhibitory response model Effect compartment model Physiological indirect model Mechanism-based agonist–antagonist interaction model
PD model	Lineal model Log-lineal model Sigmoidal E_{max} model Modified E_{max} model
PD: Pharmacodynamic; PK: Pharmacokinetic.

Figure 1. Effect compartment model applied to β -blockers.

EC_{50} : Effective concentration to yield half-maximal response; E_{max} : Maximal efficacy; k_{12} : Rate transfer constant from the central to peripheral compartment; k_{21} : Rate transfer constant from the peripheral to central compartment; k_{1e} : Rate constant for transport through the effect compartment; k_{e0} : Rate constant for the loss from the effect compartment; k_{el} : Constant of loss from the central compartment.

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Chinese volunteers [35]. According to the analysis of the Akaike's information criterion values, a goodness of fit parameter, the authors have demonstrated that the effect-compartment model provided more better-fitting PK/PD characteristics of the blood pressure lowering response of carvedilol than the indirect response model [35].

In the design of PK/PD studies of β -blockers, other aspects to consider are the biomarker of drug exposure and the pharmacological response to be evaluated. Total and unbound plasma levels of β -blocker can be used as PK information during the development of a PK/PD model. As the unbound concentration represents the bioactive fraction that is able to distribute to the target site, it seems a more powerful PK data for PK/PD modeling. Unbound concentrations of β -blockers can be assessed by *in vitro* ultracentrifugation or equilibrium dialysis of plasma samples [36]. An alternative approach is the use of intra-arterial or intravenous microdialysis [7]. Microdialysis is a highly attractive sampling technique for PK/PD studies, considering that it allows the simultaneous determination of unbound concentrations of drugs at several tissues and its action on biochemical and cardiovascular parameters [33]. Microdialysis consists in the implantation of a microdialysis probe into a tissue, that is permeable to water and small molecules. When the microdialysis probe is implanted, a perfusion media enters into the probe through the inlet tubing at a constant flow rate,

passes the dialysis membrane and is then transported through the outlet tubing and collected in a microvial [37]. While the perfusate solution passes through the dialysis membrane, unbound tissue concentrations diffuse into the perfusion fluid and are collected in dialysate samples, which are analyzed using highly sensitive techniques, such as LC and CE [37]. The most attractive aspect of this technique for PK/PD studies is the ability to allow continuous tissue sampling without removing liquid and, therefore, to provide higher temporal resolution than with traditional techniques without interfering with the PK and PD behavior of β -blockers [33]. In our laboratory, we have designed and validated a shunt intra-arterial microdialysis probe with one vascular inlet and two vascular outlets for the study of PK/PD models of antihypertensive drugs [38,39]. The inlet and one outlet are inserted into the left carotid artery and the remaining outlet is connected to a pressure transducer, allowing the simultaneous monitoring of cardiovascular parameters. By using the intra-arterial shunt microdialysis probe, we have found a good correlation was between unbound plasma concentrations of metoprolol and its chronotropic and antihypertensive effects in both normotensive and aortic coarctated rats, suggesting the utility of microdialysis for PK–PD modeling of β -blockers [38].

As cardiovascular response to β -blockers is stereospecific, it is also important to monitor plasma concentrations of β -blockers with enantioselective methods in

Figure 2. Indirect physiological model applied to β -blockers.

IC_{50} : Inhibition constant for 50% inhibition of maximum function of K_{in} ; k_{12} : Rate transfer constant from the central to peripheral compartment; k_{21} : Rate transfer constant from the peripheral to central compartment; k_{el} : Rate constant for transport through the effect compartment; k_{e0} : Rate constant for the loss from the effect compartment; k_{el} : Constant of loss from the central compartment; k_{in} : Zero-order production rate constant; k_{out} : First-order removal rate constant; R : Response.

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order to achieve accurate estimation of PK/PD parameters. For instance, only the S-enantiomer of β -blockers possess β -blocking activity, and therefore PK/PD modeling of chronotropic effect of β -blockers needs to relate unbound S-enantiomer plasma concentration with heart rate reduction [7].

Finally, in preclinical studies, PK/PD models of β -blockers can be evaluated in anesthetized or freely moving animals. As anesthesia could modify animal physiology introducing artifacts in pharmacological studies [40], the impact of anesthetic drugs used and their dosage must be taken into account during PK/PD modeling. A PK/PD study has evaluated the impact of urethane–chloralose anesthesia on PK/PD properties of carvedilol in control rats and NG-nitro-L-arginine methyl ester (L-NAME) hypertensive animals [41]. By using an effect compartment PK/PD model, it was found that urethane–chloralose did not modify PK properties and potency of the chronotropic and hypotensive effect of carvedilol, but significantly enhanced E_{max} estimation when compared with awake animals [41]. These results suggest the need to validate anesthetic drugs for accurate PK/PD modeling of β -blockers in preclinical studies.

In human PK/PD studies that evaluate the antihypertensive response to β -blockers, it is also important to take into account the placebo response and the circadian fluctuation of blood pressure [15]. Therefore, clinical PK/PD modeling of β -blockers needs the assessment of blood pressure after placebo administration and the correction of antihypertensive response induced by β -blockers by direct subtracting the blood pressure following active drug from the placebo response or by modeling the baseline with a longitudinal spline model fitted to the placebo data by nonlinear mixed effects [42].

Preclinical PK/PD studies with β -blockers

In the last years, several preclinical PK/PD studies have evaluated the pharmacological properties of traditional and third-generation β -blockers in different laboratory animals, including rat, rabbit and dog. Major contributions of preclinical PK/PD models for β -blockers include the elucidation of factors influencing cardiovascular response to these agents, the increase in the knowledge of their enantioselective PD properties and the evidence of the relevance of sympathetic overactivity in the maintenance of the hypertensive stage (Table 3) [7].

For instance, as atenolol is a second-generation β -blocker with predominantly renal elimination, Celardo *et al.* [43] have assessed the influence of renal failure on the PK and PD profiles of atenolol in adult male rabbits on continuous peritoneal dialysis. As expected, anuria was associated with a ninefold decrease of atenolol elimination and a prolongation of drug effect during anuria [43]. Nevertheless, by using an effect compartmental model, the authors did not find differences in main PK/PD parameters, E_{max} and EC_{50} , of atenolol chronotropic effect comparing rabbits before and during renal failure [43]. These results suggest that reduction of kidney function alters atenolol PK properties without affecting their PD profile [43]. In rabbits with experimental liver failure, Bortolotti *et al.* [44] have evaluated the impact of liver injury on PK/PD properties of metoprolol, a lipophilic second-generation β -blocker that undergoes extensive biotransformation. Compared with animals with normal liver function, the presence of hepatic failure induced an increase of terminal elimination half-life of metoprolol without changes in the volume of distribution. PK/PD analysis of the effect of metoprolol

Table 3. Summary of contributions of pharmacokinetic/pharmacodynamic modeling of β -blockers.

Type of study	Application	Ref.
Preclinical PK/PD modeling	Influence of renal function on PK/PD properties of atenolol	[43].
	Impact of liver injury on PK/PD properties of metoprolol	[44]
	Influence of protein binding on chronotropic effect of S-propranolol	[45]
	Relevance of sympathetic overactivity in the maintenance of hypertension	[31–32,46]
	Contribution of sympatholytic activity of carvedilol on antihypertensive response	[32]
	Characterization of sustained-release formulations of atenolol	[47]
Clinical PK/PD modeling	Comparison of PK/PD properties of immediate release and controlled release formulations of carvedilol and metipranolol	[48,49]
	Impact of gender on stereoselective pharmacokinetics and pharmacodynamics of metoprolol	[50]
	Impact of aging on blood pressure lowering response to acebutolol and dilevalol	[51,52]
	Quantification of delay in antihypertensive response to atenolol and carvedilol	[53]
	Effect of pregnancy on PK/PD properties of labetalol	[54]
	Impact of genetic polymorphism on PK/PD properties of metoprolol	[55]

PD: Pharmacodynamic; PK: Pharmacokinetic.

on heart rate by using an effect compartment model have demonstrated that liver injury does not modify maximal chronotropic response but increases EC_{50} , suggesting that hepatic failure may reduce the potency of metoprolol [44].

Mechanism-based PK/PD models have been used for the study of the influence of plasma protein binding on PDs of β -blockers. van Steeg *et al.* [45] compared the effects of four β -blockers (atenolol, propranolol, metoprolol and timolol) on heart rate under isoprenaline-induced tachycardia in conscious rats. Using a mechanism-based agonist–antagonist interaction model, the authors have found that the *in vivo* estimates of receptor affinities diverges from the *in vitro* receptor affinity, particularly for the most highly bound drug S-propranolol [45]. Therefore, the authors concluded that plasma protein binding influences PDs of β -blockers and the free plasma concentration appear to be the best predictor of *in vivo* drug potency [45]. In a second report of the research group, the influence of alpha-1-acid glycoprotein plasma concentrations on chronotropic effect of S-propranolol was investigated in rats subjected to surgery. Alpha-1-acid glycoprotein level was significantly higher at 2 days post surgery when compared with 7 days post surgery affecting the estimation of S-propranolol *in vivo* affinity. In other words, a tenfold increase in serum alpha-1-acid glycoprotein concentration changed the estimate for *in vivo* receptor affinity of S-propranolol on the basis of total plasma concentrations from 7.0 to 30 nM [56]. These results suggest that plasma protein binding restricts the PDs of S(-)-propranolol and unbound plasma concentrations of the β -blocker need to be considered for accurate PD estimation [56].

Preclinical PK/PD models also contribute to increase the knowledge of enantioselective PD prop-

erties of β -blockers and the relevance of sympathetic overactivity in the maintenance of hypertension in different experimental models. PK/PD properties of carvedilol have been compared in L-NAME hypertensive rats and normotensive rats by means enantioselective PK–PD modeling [31]. While S-carvedilol plasma levels were correlated with the chronotropic response by using an effect compartmental model, total racemic concentrations of carvedilol were related with the blood pressure lowering response considering that the R-enantiomer shows α -blocking activity [31]. Although the PK/PD parameters of the S-carvedilol chronotropic effect, including E_{max} and EC_{50} , were not altered in L-NAME rats when compared with normotensive animals, hypertensive rats showed greater potency and efficacy to the carvedilol hypotensive response, which may be explained by the greater potency of carvedilol for sympathetic vascular tone inhibition [31].

In a similar PK/PD study, the enantioselective PK/PD properties of carvedilol were studied in spontaneously hypertensive rats and Wistar Kyoto normotensive animals [46]. By using an effect compartmental model, heart rate changes induced by S-carvedilol showed a greater maximal bradycardic response in spontaneously hypertensive rats compared with the normotensive group [46]. In addition, PK/PD analysis of the hypotensive response induced by carvedilol showed a greater maximal response to racemic carvedilol in spontaneously hypertensive rats with regard to Wistar Kyoto animals [46]. Taking together, these results suggest that sympathetic overactivity contributes in both regulation of heart rate and blood pressure in spontaneously hypertensive rats [46].

In a third report, we have compared the PK/PD properties of carvedilol in fructose fed hypertensive rats, an animal model of metabolic syndrome [32]. By

adjusting to an enantioselective effect compartmental model, PK/PD parameters of the chronotropic response induced by S-carvedilol were comparable between fructose fed rats and normotensive control animals [32]. In addition, a greater maximal response with no change in potency were revealed for the hypotensive response produced by racemic carvedilol in fructose-fed rats after administration of the higher dose [32]. Taking together the results of PK/PD studies of carvedilol in different experimental models of hypertension, it seems that the contribution of the sympathetic nervous system to the hypertensive stage differs between the experimental models, being greater in spontaneously hypertensive and L-NAME rats when compared with fructose fed rats. Therefore, PK/PD modeling of the hypotensive response to β -blockers can contribute to the identification of the most sensitive population to the beneficial effects of this therapeutic group.

Recently, we have demonstrated that PK/PD modeling can contribute to establish the relevance of attenuation of vascular sympathetic activity in the blood pressure lowering response to third-generation β -blockers in experimental models of hypertension. In this context, the spectral analysis of continuous blood pressure recording offers the opportunity for studying the relationship between vasodilating β -blockers plasma levels and their effect on vascular sympathetic activity. Blood pressure shows rapid beat-to-beat oscillation due to the interplay of different cardiovascular control systems, including the baroreceptor reflex, the renin–angiotensin system, the vascular myogenic response and the release of NO from the endothelium [57]. As the response times at which different neurohormonal systems operate vary considerably, the spectral analysis of beat-to-beat blood pressure variability allows the estimation of the relative contribution of neurohumoral systems in blood pressure regulation. Spectral analysis of blood pressure recording in rats has established that variability at the low frequency (LF) domain is affected by vascular sympathetic tone and endothelial-derived NO and the normalization of LF by high frequency (HF) variability (LF/HF ratio) represents a validated marker of sympathetic vascular activity in preclinical and clinical studies [57–60]. By using an inhibitory indirect physiological model, we have found a good relationship between racemic carvedilol plasma concentrations and their effect on the LF/HF ratio, suggesting that PK/PD modeling could be a powerful tool for the assessment of the effects of third-generation β -blockers on sympathetic vascular activity [31,32]. In a previous study, a greater sympatholytic activity of carvedilol was found in L-NAME treated rats than in control normotensive rats, evidenced by a lower concentration that produces 50% of vascular sympathetic tone

inhibition (IC_{50}) in the hypertensive group [31]. Conversely, no changes in PK/PD parameters for carvedilol effect on LF/HF ratio have been found in fructose fed rats when compared with normotensive animals [32]. Taking together, PK/PD modeling of the vascular sympatholytic activity of third-generation β -blockers suggests that the contribution of vascular sympathetic overactivity in the maintenance of hypertension differs between animal models of disease.

Preclinical PK/PD modeling of β -blockers have also been used to characterize sustained-release formulations [47]. In this context, Jia *et al.* [47] have evaluated the PK/PD relationships of atenolol sustained-release pellets and immediate-release pellets by means of an effect compartmental model. A good correlation between plasma concentration of atenolol and the hypotensive effect was found in hypertensive animals after administration of both formulations. Nevertheless, estimated PK/PD parameters differ greatly between both formulation, considering that E_{max} , EC_{50} and $t_{1/2eq}$ were significantly lower after administration of sustained-release pellets when compared with the immediate-release pellets [47]. The authors concluded that the distribution process of atenolol between effect-compartment and central compartment was much faster for sustained release pellets than that for immediate-release formulation, and therefore, sustained release pellets might be a desirable delivery vehicle of cardiovascular drugs [47].

More recently, Snelder *et al.* have developed a mechanism-based PK/PD model for the characterization of the effects of cardiovascular drugs with different mechanisms of action, including β -blockers, on the interrelationship between mean arterial pressure (MAP), CO and total peripheral resistance (TPR) (Figure 3) [61]. Considering that $MAP = CO \times TPR$, the model includes two indirect physiological models to describe the time course of change in CO and 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 (Figure 3). In addition, two feedback constants are introduced in order to take into account the magnitude of the negative feedback of MAP on CO and TPR (Figure 3) [61]. During the development of the novel mechanism based PK/PD model, the authors consider that antihypertensive drugs selectively influence either CO or TPR and that all compounds influence the production rates of CO or TPR rather than the dissipation rates [61]. As β -blockers reduce stimulation of left ventricular contractility and heart rate induced by cardiac sympathetic activation, these agents are thought to influence the production rather than the dissipation rate [61]. The authors have reported that the developed mechanism-based

PK/PD model can be used for the quantification and prediction of propranolol. The proposed model may predict the effects of a particular β -blocker on blood pressure based on preclinical data [61]. Although the mechanism-based PK/PD model seems to be suitable for the evaluation of cardiovascular actions of most β -blockers, it requires modifications for vasodilatory β -blockers, considering the fact that the blood pressure reduction induced by these agents depends not only on the effects on CO but also on TPR (Figure 3).

Clinical PK/PD studies with β -blockers

PK/PD modeling of β -blockers has been used in clinical practice for the prediction of PD properties of β -blockers in patients with hypertension, mild-to-severe heart failure and in subjects after myocardial infarction with left ventricular dysfunction (Table 3). For instance, Tenero and coworkers [48] compared the PD properties the PK/PD properties of immediate release and controlled release formulations of carvedilol on change in exercise-induced heart rate in patients with cardiac dysfunction or post myocardial infarction. A good correlation was found between S-carvedilol plasma concentrations and the chronotropic response using a direct effect inhibitory $E_{\max}$ model [48]. The PK/PD model defined in healthy subjects was able to predict heart rate effects of carvedilol in patients with

mild-to-severe heart failure or asymptomatic patients with post myocardial infarction treated with immediate and controlled release formulations [48]. The area under the effect curve after controlled release carvedilol was equivalent to immediate release formulation, suggesting a 24-h β -blocking coverage for the controlled release formulation of carvedilol given once daily in patients with heart failure [48]. This study clearly demonstrates the relevance of PK/PD modeling for the optimization of dose regimen of β -blockers in patients with heart failure.

PK/PD modeling of chronotropic and hypotensive effect of metoprolol in healthy subjects has been used to establish the impact of gender on stereoselective PKs and PDs of metoprolol [50]. The authors established that the PD data were best fitted by the Hill equation with the effect site in the central compartment, rather than the effect compartment. Both maximal blood pressure lowering response and potency of metoprolol do not differ between men and women, concluding that the greater antihypertensive response to metoprolol in female subjects is due to gender-specific differences in metoprolol PK [50].

The effect of aging on pharmacological properties of acebutolol was studied in thirty subjects by means of PK/PD modeling [51]. After administration of a single intravenous dose of acebutolol, a significant

Figure 3. Mechanism based pharmacokinetic/pharmacodynamic modeling of β -blockers according to the model proposed by Snelder *et al.* [61]. MAP is determined by both physiological functions: CO and TPR. Traditional β -blockers reduce MAP by inhibiting the production of CO by myocardial β_1 -adrenergic antagonism. Vasodilatory β -blockers reduce MAP by inhibiting both the production of CO and the production of TPR due its ability to induce relaxation of vascular smooth muscle.

CO: Cardiac output; MAP: Mean arterial pressure; TPR: Total peripheral resistance.

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relationship was found between plasma drug concentrations and the cardiovascular response. Although aging did not modify acebutolol disposition, a significant reduction of blood pressure lowering response to acebutolol was found with increasing age [51].

The relationship between plasma levels of the second- and third-generation β -blockers atenolol and carvedilol and their hypotensive effects has been investigated by means of an effect compartment PK/PD model [53]. By adjusting the effect concentrations of both β -blockers with blood pressure reduction using an $E_{\max}$ model, the authors have found a significant delay in the onset of hypotensive response in healthy volunteers. This findings suggest that PK/PD modeling is able to predict the delay in the onset of the pharmacological effect of β -blockers optimizing time of antihypertensive drug dosing. It is well known that blood pressure fluctuates according to the time of the day, rising rapidly in the morning upon awakening [12]. Therefore, time of dosing of antihypertensive drug is essential to improve treatment of hypertension. Nowadays, optimal time dosing of antihypertensive drugs is selected considering the PK profile of the drug rather than its blood pressure lowering response [15]. Taking into account the findings of PK/PD studies, in the case of β -blockers, the delay in the onset of action determined by PK/PD modeling must be added to the delay in achieving maximal plasma concentration of these drugs after oral dosing in order to estimate optimal time of dosing.

PK/PD modeling has also been used to describe PK and PD properties of labetalol in pregnant women [54]. As labetalol is frequently prescribed for the treatment of hypertension during pregnancy, the influence of this physiological state on labetalol PK and PD properties is clinically relevant [54]. Saotome *et al.* [54] have described the relationship between labetalol plasma levels and the hypotensive response was described in pregnant women with moderate-to-severe hypertension during the third trimester by using a sigmoidal $E_{\max}$ model. The authors found a three- to fivefold intersubject variability in the PD parameters $E_{\max}$ and EC_{50} of labetalol hypotensive response, demonstrating the relevance of PK/PD studies for the evaluation of the variability in antihypertensive drug response and optimization of dose selection [62].

The effects of genetic polymorphism on PK/PD properties of β -blockers have also been established using a PK/PD modeling approach. In this context, Jonkers and coworkers compared PK/PD parameters of metoprolol antagonism of terbutaline-induced hypokalemia – an *in vivo* parameter of β_2 -adrenoceptor blockade – in both extensive and poor metabolizers [55]. By using an effect compartment PK/PD model,

the authors have found a higher IC_{50} of racemic and S-metoprolol in poor metabolizers when compared with extensive metabolizers [55]. Taking into account these findings, differences in metoprolol β_2 -adrenergic blockade potency between extensive and poor metabolizers can be attributed to differences in metoprolol disposition [55].

In another clinical PK/PD study, Koopmans *et al.* [63] have evaluated the effects of oxprenolol on heart rate and blood pressure using a noninvasive technique for continuous measurement of blood pressure. A good temporal relationship has been found between oxprenolol plasma concentrations after oral administration and their effect on heart rate and blood pressure during physical exercise in healthy volunteers [63]. The effect of oxprenolol on systolic blood pressure and heart rate during exercise has shown a good correlation with plasma concentrations using an $E_{\max}$ model [63].

PK/PD modeling has also been used to compare the PK and PD properties of conventional and controlled-release formulations of metipranolol in healthy men [49]. The authors have found a good relationship between plasma drug concentration of deacetylmecipranolol, the active metabolite of metipranolol, after oral administration of conventional and controlled-release tablets and the inhibition of exercise-induced tachycardia by means of a direct $E_{\max}$ model [49]. The controlled-release formulation of metipranolol has produced a relative constant plasma concentration of deacetylmecipranolol reducing the fluctuation in heart rate changes [49].

Finally, Macphee *et al.* [52] have used a PK/PD modeling approach to investigate the influence of age on antihypertensive response to dilevalol, a nonselective β -adrenoceptor antagonist with vasodilator properties, in 18 patients with essential hypertension. By using an effect compartment PK/PD model, the authors have found that aging do not influence in main PK/PD parameters of dilevalol, including $E_{\max}$ and EC_{50} [52]. These results oppose to the concerns that β -blockers might have reduced efficacy in the elderly [52].

Although PK/PD modeling of β -blocker may have a great impact in drug development and clinical practice, there are relative few clinical studies that use this approach to increase the knowledge of pharmacological properties of these cardiovascular drugs. The need of simultaneous assessment of plasma drug concentration and the cardiovascular response at several time points can hamper the clinical use of PK/PD modeling [15]. Population PKs, based on sparse plasma sampling and the PK analysis of plasma concentrations by means of the nonlinear mixed-effect modeling, are increasingly used for the evaluation of PK properties of β -blockers in clinical practice (for

review see [7]). Although to date no population PK/PD models has been developed for β -blockers, this approach could increase the applicability of PK/PD modeling for clinical studies of β -blockers. In this context, Tanigawara *et al.* have successfully designed a population PK/PD model for the evaluation of the antihypertensive response to olmesartan and azelnidipine in essential hypertensive patients [64]. Blood samples were taken a just prior to the last dose of the corresponding antihypertensive agent and 1, 2, 4, 8 and 24 h after the last dose concomitantly with the 24-h ambulatory blood pressure measurement. The authors found a good correlation between plasma concentrations of olmesartan and azelnidipine and their antihypertensive response by using an $E_{\max}$ model and established that pretreatment plasma renin activity could be a valid biomarker for cardiovascular response to olmesartan [64].

Conclusion

PK/PD modeling represents an attractive approach for a more comprehensive evaluation of preclinical and clinical pharmacological properties of β -blockers. Taking into account the great diversity in the PK and PD behavior of agents with β -blocking activity, PK/PD modeling could be of great value to identify the optimal characteristics of a β -blocker for the treatment of different cardiovascular conditions. Preclinical and clinical studies using PK/PD models have been able to identify different factors associated with cardiovascular response of β -blockers, including age, pharmaceutical formulation and genetic polymorphism, among others. Therefore, PK/PD modeling of β -blockers can be attractive for the early detection of poor responders and the optimization of dose regimen in their different indications.

Future perspective

Analysis of the evidence has shown that PK/PD modeling studies contribute to the actual knowledge of pharmacological properties of β -blockers mainly in the field of hypertension. In the next years, preclinical PK/PD models can be used to predict the clinical efficacy of β -blockers in different hypertensive stage helping in the individualization of the treatment of hypertension. The availability of different animal models that mimics the diverse forms of human hypertension may contribute to the identification of the most sensitive hypertensive population. For instance, PK/PD modeling of hypotensive effect has revealed an increased sensitivity to blood pressure reduction of carvedilol in fructose fed rats, suggesting that β -blockers could be an attractive approach for the management of hypertension in patients with metabolic syndrome [32]. Pre-

clinical PK/PD modeling could also contribute to increase gain of information during preliminary stages of drug development of potential new β -blockers [15]. As PK/PD modeling allows the estimation of *in vivo* CE_{50} , this strategy provides a precise definition of the dose–concentration–pharmacological effects and dose–concentration–toxicity relationship in early phase of drug development and serves to establish the appropriate dosing regimen for Phase I studies of investigational β -blockers [15]. There is also a potential of preclinical PK/PD modeling, especially mechanism based models, to identify biomarkers of drug response to β -blockers in animal models for efficacy and toxicity [15]. For instance, although further studies are needed, LF/HF ratio can be considered a biomarker of hypotensive response of vasodilating β -blockers in animal models of hypertension.

At the clinical setting, PK/PD modeling has the potential to contribute in the early detection of poor responders. One of the main challenges in the management of hypertension is the early selection of the most effective antihypertensive agent and its ideal dosing regimen [15]. Till date, no clear determinants of drug response to antihypertensive drugs have been discovered, making the selection of the initial therapy mainly empirical. Different PK/PD modeling studies have established that the responsiveness to the first dose of antihypertensive drugs correlates with the pharmacological response of the drug at the steady state, suggesting that the acute hypotensive response may be a useful predictor of the response during chronic therapy [26]. Therefore, the assessment of PK/PD parameters of β -blockers after the first dose can contribute to the early detection of poor or nonresponders and to individualize dose requirements for optimum long-term blood pressure control [26]. Taking into account that PK/PD modeling quantifies the delay on drug response induced by β -blockers, it allows identifying the optimal time of drug dosing in order to match blood pressure reduction induced by the drug with the circadian fluctuation of blood pressure [15]. Finally, clinical PK/PD modeling of β -blockers can also contribute to the evaluation of the clinical impact of drug interactions. It is well known that several drug interactions may affect the blood pressure lowering effect of β -blockers by both PD and PK mechanisms [65]. Using PK/PD modeling for the estimation of relevant PK/PD parameters, this strategy can potentially assess the mechanism involved in the drug interaction and its clinical relevance [15].

In the next years, it is expected that PK/PD modeling of β -blockers can evolve by the development of population PK/PD models and more sophisticated models that include parameters of disease progression

and the inclusion of tissue concentrations in the model rather than plasma levels. Physiologically based PK (PBPk) models have been developed in order to predict tissue PK of β -blockers. Although no PBPk/PD models have been developed to date for β -blockers, experience in other fields suggests the utility of this approach. For instance, recently a PBPk/PD model has been designed for the prediction of the impact of genetic polymorphisms on PK and PD of central acting drugs [66].

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Executive summary

Background

- European guidelines continue to consider β -blockers as first-line therapy of hypertension, stable coronary artery disease, systolic heart failure and ventricular arrhythmias.
- β -blockers differ in the receptor selectivity, partial or inverse agonism, membrane stabilizing effect and presence of additional vasodilatory properties.
- The great diversity of pharmacokinetic (PK) and pharmacodynamic (PD) properties of the available β -blockers highlights the relevance of the extensive evaluation of PK and PD properties of these agents in order to optimize the outcomes of the treatment of cardiovascular diseases.

Rationale of PK/PD modeling for β -blockers

- PK/PD models build a bridge between the time course of drug concentrations of β -blockers and the magnitude of the observed pharmacological response allowing a more comprehensive evaluation of pharmacological properties.
- Different PK/PD models have been developed to describe the cardiovascular actions of β -blockers, mainly the effect compartment model and the indirect physiological model.

Contribution of PK/PD modeling for β -blockers

- Major contributions of preclinical PK/PD models for β -blockers include the elucidation of factors influencing cardiovascular response to these agents, the increase in the knowledge of their enantioselective PD properties and the evidence of the relevance of sympathetic overactivity in the maintenance of the hypertensive stage.
- PK/PD modeling of β -blockers has been used in clinical practice for the prediction of PD properties of β -blockers in patients with hypertension, mild-to-severe heart failure and in subjects after myocardial infarction with left ventricular dysfunction.

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