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ORIGINAL ARTICLE

Pharmacokinetic-pharmacodynamic (PK-PD) modeling of cardiovascular effects of metoprolol in spontaneously hypertensive rats: a microdialysis study

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Abstract The present work addressed possible alterations in the pharmacokinetics and the in vivo pharmacodynamic of metoprolol (MET) in spontaneously hypertensive (SH) rats and Wistar Kyoto (WKY) animals by means of the microdialysis technique. The correlation between MET unbound plasma concentrations and its pharmacological effects, such as heart rate and blood pressure change, was also examined in SH and WKY rats by the application of a PK-PD model. MET dialysate concentrations and its chronotropic and blood pressure effect were determined during 3 h after the administration of 3 and 10 mg.kg^{-1} of the drug. A PK-PD model with a separate effect compartment was used to analyse the data. A good correlation between plasma MET concentrations and its hypotensive and chronotropic effect was found in all experimental groups. Although a greater maximal effect (E_{max}) for the antihypertensive effect of MET was observed in SH rats (WKY: E_{max}: −17±1 mmHg; SH: E_{max}: −28±4 mmHg; $P<0.05$ versus WKY rats), no differences were found in the concentration yielding half-maximal response (IC_{50}) comparing SH (IC_{50}: 583±146 ng.ml^{-1}) and WKY animals (IC_{50}: 639±187 ng.ml^{-1}). The bradycardic effect of MET was greater in SH rats (E_{max}: −29±1%, $P<0.05$ versus WKY rats) than in WK animals (E_{max}: −22±2%), but no differences were observed in the IC_{50} comparing both experimental groups (WKY: IC_{50}: 187±53 ng.ml^{-1}; SH: IC_{50}: 216±62 ng.ml^{-1}). Pharmacokinetic analysis shows that the volume of distribution of MET was greater in SH rats (Vd: 3.4±0.5 l, $P<0.05$ versus WKY rats) with regard to Wistar Kyoto (WKY) animals (Vd: 1.9±0.2 l). The results suggest that the pharmacokinetic behaviour of metoprolol are modified in SH rats, resulting in an increased volume of distribution. A greater maximal efficacy to the hypotensive effect of metoprolol was observed in SH rats, suggesting participation of β-adrenoceptors in the maintenance of the hypertension. Also, a greater chronotropic response to metoprolol was found in the hypertensive group compared with WKY animals, suggesting that, at least in part, the greater cardiac effect of metoprolol explained the enhanced hypotensive response of the beta blocker in the SH animals.

Keywords Spontaneously hypertension · Microdialysis · Pharmacokinetic-pharmacodynamic modeling · Metoprolol · Chronotropic effect · Blood pressure

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Introduction

Two different approaches exist for characterising the dose (concentration)-effect relationship of a drug, namely the classical dose response trial and pharmacokinetic-pharmacodynamic (PK-PD) modeling (Toutain 2002). PK-PD modeling allows a deeper understanding of the action of a drug, and therefore serves to define the adequate dose regimen (Toutain 2002). Also, PK-PD modeling permits the study of the physiopathological mechanisms of experimental hypertension (Höcht et al. 2005). Finally, PK-PD modeling permits the study of the existence of a time delay in the onset of the pharmacological effect of a drug. It is well known that blood pressure varies according to the time of the day, rising rapidly in the morning upon awakening (Giles 2006). Therefore, time of dosing of antihypertensive drug is essential to improve treatment of hypertension.

One disadvantage of the PK-PD modeling is the need for simultaneous measurement of drug tissue levels and corresponding pharmacological effects at multiple time points (Toutain 2002). Blood sampling, which has traditionally been used for this purpose, provides the complication that removal of the samples themselves can interfere with pharmacokinetic and pharmacodynamic drug behavior (Elmquist and Sawchuk 1997). Microdialysis sampling provides a means of continuous plasma sampling without repeated invasive sampling, and its applicability to the study of drug metabolism and pharmacokinetics in rats has been demonstrated in several reports (Chen and Steger 1993; Elmquist and Sawchuk 1997). The possibility of microdialysis sampling without fluid loss makes this technique useful for the study of pharmacokinetic-pharmacodynamic correlations. Since the animal response is not altered by fluid loss and the microdialysis technique monitors unbound drug concentration, it is possible to study the relationship between the bioactive drug fraction and the cardiovascular response (Höcht et al. 2005).

Metoprolol is a cardioselective β-adrenoceptor blocker with inverse agonist activity (Hoffmann et al. 2004) used in the treatment of several cardiovascular pathologies such as hypertension and heart failure.

Despite its long use, the pharmacodynamic relationships of metoprolol have not been well characterised. Studies assessing the concentration-response relationship of β-adrenoceptor blockers with respect to β-adrenergic blockade have shown an excellent correlation of serum concentration with the reduction in heart rate (Zacest and Koch-Weser 1972; Conway et al. 1976; Kendall et al. 1977). However, only few studies have been made in models of hypertension such as spontaneously hypertensive rats (Antonaccio et al. 1986; Brynne et al. 1998). In previous works, we found a greater maximal efficacy to the chronotropic effect of metoprolol in coarctated rats at an early stage of hypertension (Höcht et al. 2005), but not in chronic hypertensive animals by aortic coarctation (Höcht et al. 2004a). Although other authors have previously studied PK-PD models for the chronotropic effect of metoprolol in SH rats, to the best of our knowledge, comparative studies between SH rats and normotensive animals are lacking.

A poor concentration-response relationship with regard to the hypotensive effect of metoprolol has been found in some studies (von Bahr et al. 1976; Myers and Thiessen 1980; Sklar et al. 1982), but not in others (Leonetti et al. 1975; Esler et al. 1977). The suggestion that there is no relationship between plasma levels of antihypertensive drugs and its effect on blood pressure reflects an inadequacy or failure in the approaches designed to detect such correlation. A number of factors have hampered the possible identification of a correlation, including failure to study individual patients, inability to collect sufficient pharmacodynamic data, failure to identify and account for temporal delay in the onset of the pharmacological effect, the use of restricted concentration ranges, and the use of dose rather than concentration (Meredith 1997; Brynne et al. 1998).

In a previous paper, PK-PD modeling of metoprolol hypotensive effects demonstrated a greater sensitivity to the pharmacological action in chronic aortic coarctated rats with regards to sham operated animals (Höcht et al. 2004a). To the best our knowledge, there are no described PK-PD studies of the antihypertensive effect of metoprolol in SH rats.

Therefore, the aim of this work was to study the pharmacokinetics and the in vivo pharmacodynamic of metoprolol in spontaneously hypertensive rats and Wistar Kyotto animals by means of the microdialysis technique. In addition, the correlation between metoprolol unbound plasma concentrations and its pharmacological effects, such as heart rate and blood pressure change, was also examined in SH and WKY rats by the application of a PK-PD model.

Materials and methods

Experimental procedure

Eight-week-old male spontaneously hypertensive (SH) and Wistar Kyoto (WKY) rats weighing 200–220 g were used. Animal experiments were performed in accordance with the *Principles of Laboratory Animal Care* (NIH publication no. 85–23, revised 1985).

Experiments were performed on animals anaesthetised with a mixture of chloralose (50 $mg.kg^{-1}$, IP) and urethane (500 $mg.kg^{-1}$, IP). A femoral vein was cannulated for the intravenous administration of metoprolol isotonic solution at doses of 3 and 10 $mg.kg^{-1}$. A validated "shunt" microdialysis probe with one vascular inlet and two vascular outlets (Opezzo et al. 2001; Höcht et al. 2004b) was used for examining the time course of the plasma concentrations of metoprolol. The inlet and vascular outlet of the heparinized probe (50 $IU.ml^{-1}$) were inserted in the left carotid artery, while the remaining vascular outlet was connected to a Statham Gould P23ID pressure transducer coupled to a Grass 79D polygraph. Mean arterial pressure (MAP) was calculated as the sum of the diastolic pressure and one-third of the pulse pressure. The heart rate (HR) was calculated tachographically by counting the pulsatile waves of arterial pressure recording.

Microdialysis probe was perfused at 2 $\mu l.min^{-1}$ with a solution of NaCl 147 mM, $CaCl_2$ 4 mM, KCl 4 mM, pH 7.3, using a perfusion pump. Samples were collected at 15 min intervals. The in vivo recovery of metoprolol from tissue to the perfusion medium in the dialysis probe was

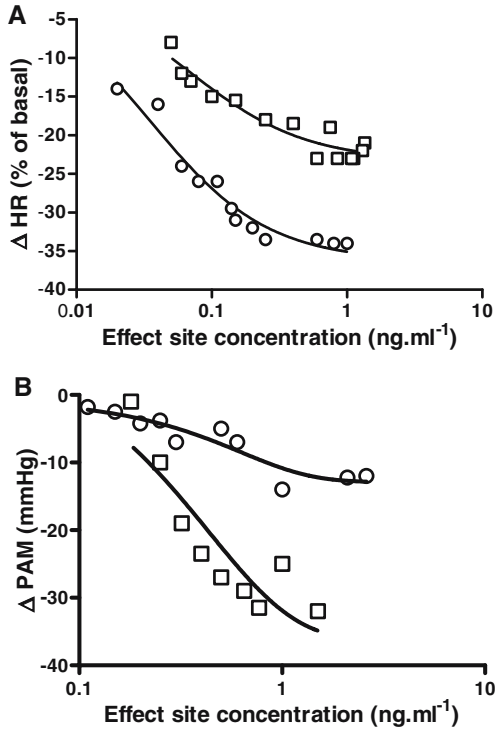

Fig. 1 Change of heart rate (**a**) and blood pressure (**b**) as a function of metoprolol effect site concentrations in a representative Wistar Kyoto (WKY, *circles*) and spontaneously hypertensive (SH, *squares*) rat

determined before intravenous injection by perfusing the microdialysis probe with a solution of metoprolol (200 ng. ml^{-1}) and by taking the proportion of lost across the dialysis membrane as an estimate of the recovery.

The in vivo recovery of metoprolol was calculated with the following equation:

$$R = (C_{in} - C_{dial})/C_{in}$$

where R is the metoprolol in vivo recovery, C_{in} is the concentration of metoprolol in the perfusate and C_{out} is the concentration of metoprolol in the dialysate. Recovery of metoprolol in all experiment was 0.24±0.08.

After determination of the in vivo recovery, an equilibration period of 30 min preceded metoprolol administration.

Metoprolol levels were determined by HPLC and fluorometric detection using a Phenomenex Luna 5-μm, C18, 250×4.60 mm column. The excitation and emission wavelengths were 228 nm and 382 nm, respectively. Composition of the mobile phase was distilled water-acetonitrile-triethanolamine (83:15:1.2), adjusted to pH 3.0 with phosphoric acid. The coefficient of variation of the chromatographic method was less than 5% and the lower limit of detection of metoprolol was 1.0 $ng.ml^{-1}$. Intra-day and inter-day coefficient of variation was 3.5 and 4.8, respectively. Accuracy ranged from 98.9 to 103.5%. The method was linear over the concentration range of 1–5000 $ng.ml^{-1}$.

Analysis of data

Pharmacokinetics of metoprolol

To determine blood unbound concentrations of metoprolol from microdialysis data, concentrations of the drug in microdialysis samples were adjusted with the in vivo recovery of the probe.

Microdialysis generated data that are the integral of the concentration surrounding the probe during the sampling interval. Therefore, the microdialysis data must to be transformed from a series of integrals to a series of points corresponding at the end time of the sample interval.

First, it is necessary to calculate the time point during the sample interval at which the mean microdialysis sample concentration is attained (T) using the following equation developed by Ståhle (1992):

$$T = \left[ln\left(k\Delta t\right) - ln\left(1 - e^{-k\Delta t}\right)\right]/k$$

where Δt is the sample interval and k the rate constant of elimination.

Next, to calculate the concentration of metoprolol in the microdialysis sample at the end time of the sample interval, the following equation was used:

$$C_{(0)} = C_{(0)}e^{-k(\Delta t - T)}$$

where $C_{(t)}$ is the concentration of metoprolol at the end of the sample interval, $C_{(0)}$ is the mean concentration of metoprolol in the microdialysis sample, Δt is the sample interval and T is the time point during the sample interval at which the mean microdialysis samples concentration is attained.

Metoprolol concentration-time profile obtained from corrected microdialysis data following bolus dosing was described by a one-compartment, first-order elimination model. Non-linear, least-squares regression analysis was performed using the TOPFIT program (version 2.0; Dr Karl Thomae Gmbh, Schering AG, Gödecke AG, Germany). The area under the curve (AUC) of the function relating metoprolol levels to time was calculated by using the trapezoidal rule. The parameters clearance (Cl) and volume of distribution (Vd) were calculated by standard methods (Gibaldi and Perrier 1982), where Vd=D / Cmax (Cmax is the maximal plasma concentration) and Cl=KexVd (Ke is the rate constant of elimination).

Pharmacokinetic-pharmacodynamic relationship of metoprolol

In the pharmacokinetic-pharmacodynamic relationship study of metoprolol, metoprolol concentrations in serum dialysate and blood pressure and heart rate change were used. As a time delay between the plasma concentrations and its cardiovascular effects was observed, a pharmacoki-

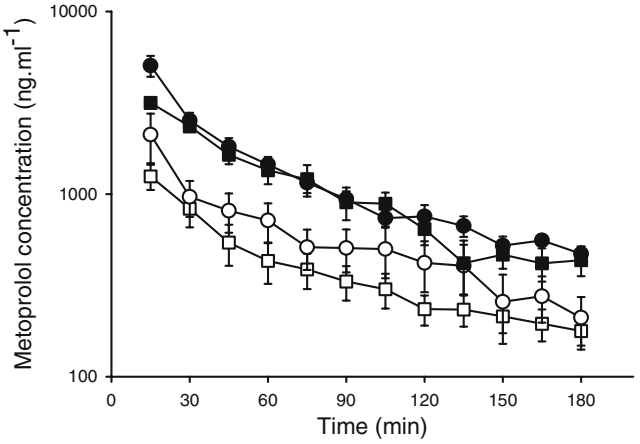

Fig. 2 Mean metoprolol concentrations in plasma dialysate of WKY (*circles*) and SH (*squares*) animals as a function of time after IV administration of metoprolol (3 mg.kg^{-1}, *open symbols*; 10 mg.kg^{-1}, *black symbols*). Each point shows the mean±SEM of six rats

netic-pharmacodynamic model with a separated effect compartment was used for the analysis of the data. Figure 1 shows the relationship between the chronotropic effect (A) and the blood pressure effect (B) of metoprolol and its effect site concentrations in representative animals from the WKY and SH group. The equation that describes the effect-site concentration (Ce) of one compartment pharmacokinetic model is:

$$Ce = D^{*}K_{e0}/Vd^{*}[e^{-kt}/(K_{e0}-K) + e^{-Ke0^{*}t}/(K-K_{e0})]$$

where D is the dose, Vd the volume of distribution, Ke0 the equilibration rate constant, t the time and K the exponential rate constant.

A non-linear regression of these data was carried out using the computer program TOPFIT by means of the following equation:

$$Y = E_{max}{}^{*}Ce(t)/(IC_{50} + Ce(t)),$$

where Y is the blood pressure change (ΔMAP, in mmHg) or the chronotropic effect (% of basal heart rate), E_{max} the maximal response, IC_{50} the metoprolol concentration yielding half maximal response and Ce the metoprolol concentration in the effect compartment at t time.

The following parameters of the pharmacokinetic-pharmacodynamic model were evaluated: IC_{50}, E_{max} and $t_{1/2\ eq}$. The parameter $t_{1/2\ eq}$ is the equilibration half time between the plasma and the effect compartment and is equal to $\ln 2/K_{e0}$.

Statistics

Normal distribution of the data and the variables of the study were verified using the Kolmogorov-Smirnov test. Data are given as mean±SEM. Statistical analysis was performed by Student's *t* test or by two-way ANOVA and the test of Bonferroni as a post hoc test. Pharmacokinetic and PK-PD parameters were log transformed for statistical analysis in order to reduce heterogeneity of the variance. Statistical analysis of pharmacokinetic and PK-PD parameters was performed by a two way ANOVA and the test of Bonferroni as the post hoc test.

Statistical tests were performed by using GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego, Calif., USA). Statistical significance was defined as $P<0.05$.

Drugs

Metoprolol was a generous gift from Novartis Lab, France.

Results

Basal values of mean arterial pressure (MAP) and heart rate (HR) were 85±8 mmHg and 353±6 bpm (n=12), respectively, in anaesthetized WKY animals and 134±9 mmHg ($P<0.05$ versus WKY rats) and 404±9 bpm. ($P<0.05$ versus WKY rats) (n=12) respectively in anaesthetized SH rats.

Table 1 Pharmacokinetic parameters of metoprolol obtained from dialisate samples from Wistar Kyoto (WKY) and spontaneously hypertensive (SH) rats after the IV administration of drug (3 mg.kg^{-1} and 10 mg.kg^{-1})

Pharmacokinetic parameter	WKY rats		SH rats	
	3 mg.kg^{-1}	10 mg.kg^{-1}	3 mg.kg^{-1}	10 mg.kg^{-1}
Cmax (μg.ml^{-1})	1.92±0.14	5.56±0.58	1.45±0.31	3.26±0.44*
AUC (ng.ml^{-1}.h^{-1})	2720±729	4768±343	1496±312	2674±355
$t_{1/2}$ (h)	0.44±0.06	0.48±0.07	0.69±0.10	0.73±0.15
Cl (ml.min^{-1})	34.4±10.1	38.4±3.4	40.0±7.5	63.2±7.9
Vd (l)	1.6±0.1	1.9±0.2	2.6±0.5	3.4±0.5*

The data were expressed as mean±SEM of six animals. (*Cmax* maximal serum concentration, *AUC* area under the curve, $t_{1/2}$ elimination half-time, *Cl* clearance, *Vd* volume of distribution)
*$P<0.05$ vs WKY rats

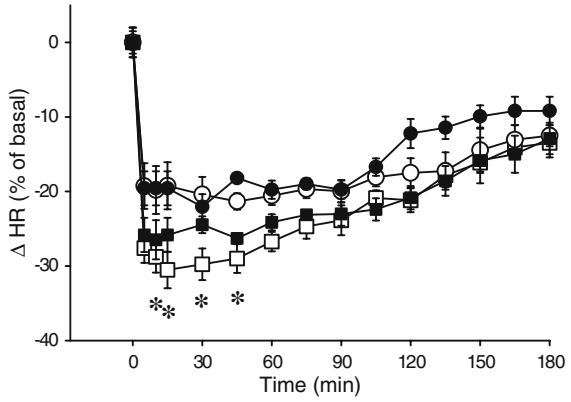

Fig. 3 Time course of the change of heart rate (ΔHR, % of basal heart rate), after IV administration of metoprolol (3 mg.kg^{-1}, *open symbols*; 10 mg.kg^{-1}, *black symbols*) in WKY (*circles*) and SH (*squares*) rats. Each point shows the mean±SEM of six animals. *P<0.05 vs WKY rats

Pharmacokinetics of metoprolol

Figure 2 shows the metoprolol concentration-time profile obtained from the microdialysis corrected data from WKY rats (n=6) and SH animals (n=6) after IV. administration (3 mg.kg^{-1} and 10 mg.kg^{-1}). The resulting pharmacokinetic parameters are shown in Table 1. Maximal serum concentration (Cmax) of metoprolol was smaller in SH rats compared to WKY rats after the administration of 10 mg.kg^{-1} of the drug. Consequently, volume of distribution (Vd) was greater in SH rats than in WKY animals at this dose level (Table 1). Although higher mean values of t1/2 were observed in SH rats, the difference was not statistically significant in comparison with WKY after the administration of 3 or 10 mg.kg^{-1} of metoprolol (Table 1). On the other hand, a non-significant greater Cl and Vd were observed in both experimental groups after the administration of the greater dose compared to the lower dose (Table 1).

Chronotropic effect of metoprolol

Figure 3 shows the time course of HR after IV administration of metoprolol (3 mg.kg^{-1} or 10 mg.kg^{-1}) in WKY (n=6) and SH rats (n=6). Change of HR was expressed as percentage of basal value during the 30 min before administration of the drug. An enhanced negative chronotropic effect was observed in SH animals (ΔHR: −30.5±2.4 bpm, n=6, P<0.05 versus WKY rats) compared to normotensive WKY rats (ΔHR: −19.2±3.2 bpm, n=6).

A good correlation between the concentration of metoprolol in the effect compartment and the pharmacodynamic data was found. E_{max} and IC_{50} for the chronotropic effect of metoprolol and the equilibration half-time ($t_{1/2,eq}$) are shown in Table 2. A greater maximal response to the bradychardic effect of metoprolol was observed in SH rats with respect to WKY animals (Table 2). On the other hand, no difference was observed in the IC_{50} parameter comparing both experimental groups (Table 2). As shown in Table 2, the equilibration half-time was similar between both experimental groups. In addition, no differences were observed in the PK-PD parameters obtained from the dose of 3 and 10 mg.kg^{-1} in both experimental groups, confirming the validity of the PK-PD analysis.

Effect of metoprolol on blood pressure

Figure 4 shows the temporary course of MAP after IV administration of metoprolol (3 mg.kg^{-1} or 10 mg.kg^{-1}) in SH and WKY rats. Although the lower dose of metoprolol reduced the MAP in both experimental groups, the maximal decrease was significant greater in SH animals (WKY rats ΔMAP: −11.6±2.1 mmHg, n=5; SH rats ΔMAP: −24.7±3.7 mmHg, n=6, P<0.05). MAP recovered to basal in both groups of animals. After administration of 10 mg.kg^{-1} of metoprolol, a similar profile of response was observed (WKY rats: ΔMAP: −14.7±2.6 mmHg, n=6; SH rats: ΔMAP: −27.3±4.3 mmHg, n=6, P<0.05).

In all cases, a good correlation between the concentration of metoprolol in the effect compartment and the pharmacodynamic data was found. E_{max}, IC_{50} and $t_{1/2\ eq}$ for the hypotensive effect (Table 3) of metoprolol were calculated. A greater maximal efficacy to the blood pressure effect of metoprolol was observed in SH rats with respect to WKY animals (Table 3). On the other hand, no difference

Table 2 Pharmacokinetic-pharmacodynamic parameters of metoprolol for the chronotropic effect of metoprolol in WKY and SH rats

Pharmacokinetic-pharmacodynamic parameter	WKY rats		SH rats	
	3 mg.kg^{-1}	10 mg.kg^{-1}	3 mg.kg^{-1}	10 mg.kg^{-1}
IC_{50} (ng.ml^{-1})	144±45	187±53	100±28	216±62
E_{max} (%)	−23±1	−22±2	−31±2*	−29±1*
$t_{1/2\ eq}$ (h)	0.61±0.07	0.34±0.06	0.45±0.09	0.37±0.17

The data were expressed as mean±SEM of six animals. (IC_{50} metoprolol concentration yielding half-maximal response, E_{max} maximal response, $t_{1/2\ eq}$ equilibration half-time between plasma and effect compartment)
*P<0.05 vs WKY rats

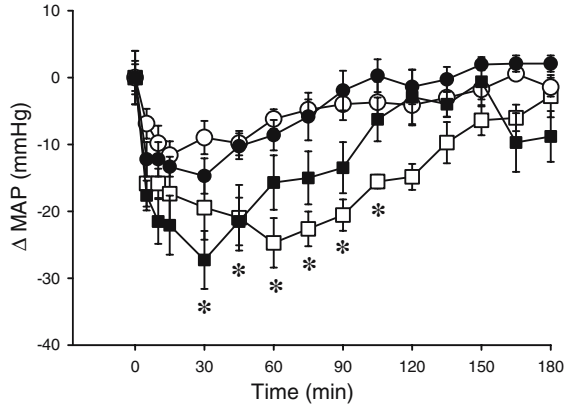

Fig. 4 Time course of the change of mean arterial pressure (ΔMAP), after IV administration of metoprolol (3 mg.kg^{-1}, *open symbols*; 10 mg.kg^{-1}, *black symbols*) in WKY (*circles*) and SH (*squares*) rats. Each point shows the mean±SEM of six animals. *P<0.05 vs WKY rats

was observed in the IC_{50} and the $t_{1/2\ eq}$ parameter comparing both experimental groups (Table 3). In addition, no differences were observed in the PK-PD parameters two doses in both experimental groups, confirming the validity of the PK-PD analysis.

Discussion

The present work addressed the relationship between unbound plasma metoprolol concentration and its cardiovascular effects in WKY and SH rats. Although PK-PD modeling has several advantages over classical dose-response studies, only few experimental PK-PD studies have been conducted for cardiovascular drugs. Study of PK-PD models of antihypertensive drugs provides mechanisms for linking dose and response (Toutain 2002). Also, comparison of PK-PD parameters obtained from experimental hypertensive models and its respective control group allows the study of the physiopathological mechanism involved in the maintenance of the hypertensive stage (Höcht et al. 2005).

To the best our knowledge, although PK-PD modeling was used to demonstrate the chronotropic effect of metoprolol in SH animals, PK-PD studies of metoprolol hypotensive effect are lacking in this experimental model of hypertension. One reason for this is the suggestion that there is no relationship between plasma levels of antihypertensive drugs and its effect on blood pressure. Also, PK-PD modeling needs to cover the complete pharmacodynamic range of a drug after a single administration (Toutain 2002). In the present work, as shown in Figs. 3 and 4, maximal response was obtained for both the chronotropic and hypotensive effect of metoprolol allowing traditional PK-PD modeling of the data.

Another disadvantage that limits PK-PD studies is the need for drug tissue concentrations and the corresponding pharmacological effect at multiple time points. In this regard, traditional blood sampling is not ideal because removal of the samples themselves interferes with the pharmacokinetic and pharmacodynamic behavior of the drug. The microdialysis technique is a powerful tool for the determination of extracellular free drug concentration for pharmacokinetic purposes in experimental animals. Advantages of this technique include: 1) frequent determinations may be made, which can provide more information about the shape of the drug concentration- time profile and allow the use of the same animals for multiple experiments, without concern for the volume of blood loss from small animals, 2) the ability to perform continuous sampling without altering drug pharmacokinetics as a result of physiological changes caused by removal of blood samples and 3) in vivo determination of unbound drug concentration in the blood can be performed (Benveniste and Huttemeier 1990; Ungerstedt 1991). In our laboratory, a shunt intraarterial microdialysis probe was designed with one vascular inlet and two vascular outlets (Opezzo et al. 2001). The inlet and one outlet are inserted into the left carotid artery and the remaining outlet is connected to a pressure transducer. This allows the simultaneous monitoring of haemodynamic variables, making the microdialysis probe a powerful tool for the study of PK-PD models of cardiovascular drugs (Höcht et al. 2005).

Table 3 Pharmacokinetic-pharmacodynamic parameters of metoprolol for the hypotensive effect of metoprolol in WKY and SH rats

Pharmacokinetic-Pharmacodynamic parameter	WKY rats		SH rats	
	3 mg.kg^{-1}	10 mg.kg^{-1}	3 mg.kg^{-1}	10 mg.kg^{-1}
IC_{50} (ng.ml^{-1})	378±192	639±187	377±122	583±146
E_{max} (%)	−15±1	−17±1	−31±4*	−28±4*
$t_{1/2\ eq}$ (h)	0.30±0.09	0.22±0.04	0.62±0.23	0.24±0.05

The data were expressed as mean±SEM of six animals. (IC_{50} metoprolol concentration yielding half-maximal response, E_{max} maximal response, $t_{1/2\ eq}$ equilibration half-time between plasma and effect compartment)
*P<0.05 vs WKY rats

The most important pharmacokinetic characteristics of metoprolol are its low plasma protein binding and its hepatic metabolism through the cytochrome family member CYP2D6 (Flockhart and Tanus-Santos 2002; Zanger et al. 2004). Although the pharmacokinetic properties of metoprolol have been studied in normotensive (Mostafavi and Foster 2000) and spontaneous hypertensive (Yin et al. 1997) animals, there are, to the best of our knowledge, no studies indicating that the hypertensive stage modifies the pharmacokinetics of the drug. In the present work, a greater volume of distribution of metoprolol was found in SH rats with regards to WKY normotensive animals, suggesting an alteration in body composition in the hypertensive group. Taking into account the lipophilicity of metoprolol, our results suggest an increase in adipose tissue of SH rats. It is well known that lipolysis induced by catecholamines is reduced in SH animals (Spitzer et al. 1985; Nelson et al. 1987; Chiappe de Cingolani 1988). Also, SH rats are considered a model of human metabolic Syndrome X, in which hypertension is associated with dyslipidemia and with insulin resistance of glucose metabolism (Hajri et al. 2001).

A linear relationship was observed between dose and Cmax of metoprolol in WKY rats. However, lower than expected Cmax values were calculated in SH rats because of a non-significant increase of volume of distribution with the highest dose. Moreover, AUC increased less than proportionally with dose in SH and WKY rats. This non-proportional relationship between AUC and dose may be explained by a non-significative increase in the clearance of metoprolol in both experimental groups. Although higher mean clearance values were calculated for SH rats the great variability observed for this parameter would no suggest an alteration in the hepatic metabolism of metoprolol in SH rats with respect to WKY animals.

In the present work, the relationship between metoprolol plasma concentrations and its bradychardic effect was studied in SH and WKY animals. Administration of 10 $mg.kg^{-1}$ of metoprolol induced a similar chronotropic effect in both experimental groups comparing to the lower dose, suggesting that the tested doses of metoprolol cover the complete pharmacodynamic range of its chronotropic effect. Previously, two PK-PD studies of the chronotropic effect of metoprolol were made in SH animals (Yin et al. 1997; Brynne et al. 1998). However, in the first place, the authors did not compare the hypertensive group with normotensive animals. On the other hand, the applied PK-PD model in the previous studies differs from the PK-PD model with an effect compartment used in the present work. In both studies, the authors directly linked plasma metoprolol concentrations to its bradychardic effect (Yin et al. 1997; Brynne et al. 1998). However, as shown in the present work, a time delay exists in the maximal chronotropic effect of metoprolol with regards to its plasma concentration.

The extent of the in vivo negative chronotropic effect of metoprolol depends on β_1-adrenoceptor sensitivity, metoprolol affinity and noradrenaline turnover. It is well known that noradrenaline cardiac release is enhanced in SH animals with respect to WKY normotensive group (Abboud 1982; Tsoporis and Leenen 1988; Adams et al. 1989). In the present work, PK-PD modeling demonstrated a greater maximal chronotropic response in the hypertensive group with respect to WKY animals without change in the IC_{50} parameter. Although basal heart rate has several limitation as a marker of cardiac sympathetic activity (Grassi 1998), the enhanced basal heart rate observed in SH animals could suggest an increased in the cardiac sympathetic activity in the hypertensive group. Therefore, our results suggested that metoprolol in vivo affinity to cardiac β_1-adrenoceptors is not altered. On the other hand, the greater chronotropic effect results from the enhanced noradrenaline release observed in SH animals with respect to normotensive WKY rats.

In the present work, the correlation between the metoprolol concentrations and its blood pressure effect was also studied. Only few reports have evaluated the blood pressure response to acute administration of metoprolol. Metoprolol significantly lowered the blood pressure in spontaneously hypertensive rats (Buckingham and Hamilton 1980; Antonaccio et al. 1986). However, there are, to the best our knowledge, no studies that compare the hypotensive effect of the beta blocker in SH animals with respect to WKY rats. In our work, the temporal course of blood pressure after two different doses of metoprolol administration demonstrated a greater hypotensive effect in hypertensive rats than in normotensive rats. No differences were observed in the PK-PD parameters comparing both dose levels in SH and WKY animals. So, we could conclude that the pharmacodynamic range of blood pressure effect of metoprolol was covered in our study.

Many mechanisms have been postulated to account for the reduction of blood pressure induced by β-adrenergic antagonist, including reduction in myocardial contractility and cardiac output, reduction in the secretion of renin, alteration of the control of the sympathetic nervous system at the level of the central nervous system, a change in baroreceptor sensitivity, an alteration in peripheral adrenergic neuron function and an increase in prostacyclin synthesis (Oates and Brown 2001).

PK-PD analysis of the hypotensive effect of metoprolol demonstrated enhanced maximal response in the hypertensive group without alteration in the IC_{50} parameter. Also, equilibration half-time between the plasma concentrations and the effect compartment concentrations of metoprolol was similar in both experimental groups, indicating that the

rate of onset of the blood pressure effect of metoprolol did not change in the SH rats. Taking into account that a central hypotensive action of metoprolol has been postulated, a possible explanation for the delay in the onset of the hypotensive effect of metoprolol could be related to permeability of the blood-brain barrier.

The greater maximal response to metoprolol hypotensive effect observed in SH animals could suggest an involvement of β-adrenoceptors in the regulation of blood pressure in this experimental model. Taking into account that the cardiac effect of metoprolol was greater in the hypertensive group compared with WKY animals, it could be suggested that, at least in part, cardiac β-adrenoceptors are implicated in the greater antihypertensive effect of metoprolol in SH rats. On the other hand, in both experimental groups, the IC_{50} for the hypotensive effect of metoprolol was greater than the IC_{50} for its chronotropic effect. Therefore, our results suggest that other additional mechanisms to the cardiac effect of metoprolol are involucrate in the antihypertensive effect of the beta blocker.

In conclusion, the results suggest that pharmacokinetic behaviour of metoprolol are modified in SH rats, resulting in an increased volume of distribution. A good correlation was found between effect compartment concentration of metoprolol and its cardiovascular effects in both experimental groups. These results demonstrated that the suggestion that there is no relationship between plasma levels of β-blockers and its effect on blood pressure is probably a consequence of an inadequate experimental design and data analysis. A greater maximal efficacy to the hypotensive effect of metoprolol was observed in SH rats, suggesting a participation of β-adrenoceptors in the maintenance of the hypertension. Also, a greater chronotropic response to metoprolol was found in the hypertensive group, suggesting that, at least in part, the greater cardiac effect of metoprolol explained the enhanced hypotensive response of the β blocker in SH animals.

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