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Original article

Applicability of microdialysis as a technique for pharmacokinetic–pharmacodynamic (PK–PD) modeling of antihypertensive beta-blockers

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Abstract

Introduction: The aim of the present work was to examine microdialysis as a technique for the study of pharmacokinetic–pharmacodynamic modeling of antihypertensive drugs. For this purpose, we studied the antihypertensive and the chronotropic effect of metoprolol and its plasma concentrations in sham operated (SO) and aortic coarctated (ACo) rats at an early hypertensive stage. **Methods:** Plasma metoprolol concentrations were obtained by means of a "shunt" vascular microdialysis probe. Changes in mean arterial pressure and heart rate were also measured in the same experiment. **Results:** A rapid decay of metoprolol levels was observed in both experimental groups. For the chronotropic effect, a good association between plasma levels and the chronotropic effect was observed in SO and ACo rats. ACo rats had a greater sensitivity to the chronotropic effect (E_{max}: $-38\pm2\%$, $n=5$, $p<0.05$) than SO animals (E_{max}: $-27\pm1\%$, $n=5$). A delay in the blood pressure reduction induced by metoprolol was observed in both experimental groups. A good association was observed between concentrations of metoprolol in the effect compartment and the corresponding hypotensive effect in both experimental groups. The calculated PK–PD parameters were not different between SO and ACo groups. **Discussion:** A good correlation was found between metoprolol concentration and its chronotropic and antihypertensive effects in normotensive and ACo hypertensive rats, allowing the employment of PK–PD models. The microdialysis technique allows simultaneous determination of plasma levels of antihypertensive drugs and their cardiovascular effects, and is therefore a powerful tool for PK–PD modeling.

Keywords: Aortic coarctation; Cardiovascular effects; Metoprolol; Microdialysis; Pharmacokinetic–pharmacodynamic models; "Shunt" vascular probe

1. 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). It is well known that the PK–PD modeling has several advantages over classical dose–response studies. PK–PD modeling not only allows better pharmacodynamic characterisation of drugs but also permits screening and dosage–regimen selection (Toutain, 2002).

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). However, 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 & Sawchuk, 1997). The development of microdialysis for the purpose of measuring drug concentration was initiated during the late 1980s (Ben-Hun, Cooper, Cringle, & Constable, 1988; Brodie, Lee, Fredholm, Ståhle, & Dunwiddie, 1987; Hurd, Kehr, & Ungersted, 1988). This

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technique also provides a means of continuous plasma sampling without repeated blood sampling and its applicability to the study of drug metabolism and pharmacokinetics in rats has been demonstrated in several reports (Chen & Steger, 1993; Elmquist & 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. Previously, by using the microdialysis technique, a correlation between the cardiovascular effect of the antihypertensive drug methyldopa and its arterial blood concentration in sinoaortic denervated and control rats was observed (Opezzo, Höcht, Taira, & Bramuglia, 2000).

In our laboratory, a shunt intraarterial microdialysis probe was designed with one vascular inlet and two vascular outlets. 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 (Opezzo et al., 2000). This probe was previously validated for the study of the plasma pharmacokinetics of antihypertensive drugs such as irbesartan (Höcht, Opezzo, & Taira, 2003).

Metoprolol is one of the most widely used drugs, being prescribed for the treatment of cardiovascular diseases as diverse as hypertension, angina pectoris, arrhythmias and recently heart failure. Despite the longevity of its use, the pharmacodynamic properties of metoprolol have not been well characterised. Studies assessing the concentration–response relationship of β-adrenoceptor blockers have shown an excellent correlation of serum concentration with the reduction of heart rate associated with β-adrenergic blockade (Conway, Fitzgerald, Mcainsh, Rowlands, & Simpson, 1976; Kendall, Brown, & Grieve, 1977; Zacest & Koch-Weser, 1972). However, a poor concentration–response relationship appears to exist with regard to their hypotensive effects (Myers & Thiessen, 1980; Sklar et al., 1982; von Bahr et al., 1976), although some investigators have found a good association (Esler, Zweifler, Randall, & DeQuattro, 1977; Leonetti et al., 1975).

The aim of the present work was to study the application of the microdialysis technique to the pharmacokinetic–pharmacodynamic (PK–PD) modeling of cardiovascular drugs such as metoprolol in normotensive sham operated animals and aortic coarctated rats (ACo) at an early hypertensive stage.

2. Methods

2.1. Induction of the hypertension

Female Wistar rats were used (220–250 g). Animal experiments were performed in accordance with the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985).

Aortic coarctation (ACo) was carried out according to the method described by Rojo-Ortega and Genest (1968) in rats anaesthetised with ether. The technique consists of banding the aorta between the two renal arteries. Control animals were sham operated (SO) rats. Experiments were carried out 7 days after the corresponding operation.

2.2. Experimental design

Experiments were performed on animals anaesthetised with a mixture of chloralose (50 mg kg^{-1}, i.p.) and urethane (500 mg kg^{-1}, i.p.). A femoral vein was cannulated for the intravenous administration of isotonic solution containing metoprolol at a dose of 3 mg kg^{-1}. A validated "shunt" microdialysis probe with one vascular inlet and two vascular outlets (Höcht et al., 2003) was used for examining the time course of metoprolol plasma concentrations. The inlet and vascular outlet of the heparinized probe (50 U ml^{-1}) were inserted into the left carotid artery, while the remainder vascular outlet was connected to a Statham Gould P23ID pressure transducer coupled to a Grass 79D polygraph.

The mean arterial pressure (MAP) was calculated according to the formula:

$$\mathrm{MAP} = \text{diastolic pressure} + (\text{systolic pressure} - \text{diastolic pressure})/3$$

The heart rate (HR) was calculated from the arterial blood pressure recording by means of a tachograph.

The microdialysis probe was perfused with a solution consisting of NaCl 147 mM, $CaCl_2$ 4 mM, KCl 4 mM at pH 7.3 using a perfusion pump. The flow rate was 2 μl min^{-1} and samples were collected at 15 min intervals. The in vivo recovery of metoprolol from the tissue to the perfusion medium in the dialysis probe was 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:

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

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 experiments was 0.25 ± 0.07.

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

Metoprolol levels were determined by high pressure liquid chromatography and fluorometric detection using a Phenomenex Luna 5 μm, C18, 250×4.60 mm column. The excitation and emission wavelength was 228 and 382 nm, respectively. The mobile phase was composed of a distilled water–acetonitrile–triethylamine mixture (83:15:1.2) adjusted to pH 3.0 with phosphoric acid.

2.3. Analysis of data

2.3.1. Metoprolol pharmacokinetics

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

The temporal profile of metoprolol concentration obtained from the 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 (A_{UC}) of metoprolol levels versus time was calculated by using the trapezoidal rule. The parameters clearance (Cl) and volume of distribution (V_d) were calculated by standard methods (Gibaldi & Perrier, 1982), where Cl=dose/A_{UC} and V_d=Cl/K_e.

2.3.2. Pharmacokinetic–pharmacodynamic modeling of metoprolol antihypertensive effect

In the metoprolol pharmacokinetic–pharmacodynamic relationship study, the serum dialysate metoprolol concentration and blood pressure change data were used. Since a time delay between the plasma concentrations and the effect was observed, a pharmacokinetic–pharmacodynamic model with a separated effect compartment was used for the analysis of the data. The equation that describes the effect-site concentration (C_e) of a one compartment pharmacokinetic model is:

$$C_e = D^{*}K_{e0}/V_d^{*}\left[e^{-Kt}/(K_{e0}-K) + e^{-K_{e0}^{*}t}/(K-K_{e0})\right]$$

where D is the dose, V_d is the volume of distribution, K_{e0} is the equilibration rate constant, t is the time and K is the exponential rate constant.

A non-linear regression was carried out on these data using the computer program TOPFIT. Data were adjusted to the following equation:

$$\Delta MAP = E_{max}{}^{*}C_e(t)/(IC_{50} + C_e(t)),$$

where ΔMAP is blood pressure change, E_{max} is the maximal response, IC_{50} is the metoprolol concentration corresponding to half maximal effect and C_e is the metoprolol concentration in the effect compartment at time t.

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

2.3.3. Pharmacokinetic–pharmacodynamic modeling of metoprolol chronotropic effect

It is necessary after drug administration for the maximal effect to be reached to apply a conventionally pharmacokinetic–pharmacodynamic (PK–PD) model (Toutain, 2002). Often in clinical pharmacology, the maximum effect of a compound will be unknown owing to safety concerns. In this case, an alternative PK–PD model for data analysis was designed by Schoemaker, van Gerven, and Cohen (1998). The authors replaced the parameter IC_{50} with S_0 in the E_{max} equation, which represents the initial sensitivity to the drug.

A non-linear regression of metoprolol plasma concentration and chronotropic effect was carried out using the computer program GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego California USA). Data were adjusted to the following equation:

$$\Delta HR = S_0{}^{*}E_{max}C_d(t)/(E_{max} + S_0{}^{*}C_d(t)),$$

where ΔHR is the change in heart rate, S_0 is the initial sensitivity to the drug, E_{max} is the maximal response and C_d is the metoprolol plasma concentration at time t.

2.4. Statistics

Normal distribution of the data and the variables of the study were verified using the Kolmogorov–Smirnov test. Data were expressed as mean±SEM of 5 animals. Statistical analysis was performed by Student's "t" test or by ANOVA and the test of Bonferroni as a post hoc test.

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

2.5. Drugs

The following drugs were used: metoprolol (a generous gift from Novartis Lab, France).

3. Results

Basal values of mean arterial pressure (MAP) and heart rate (HR) were 81±3 mmHg and 378±11 bpm (n=10) in

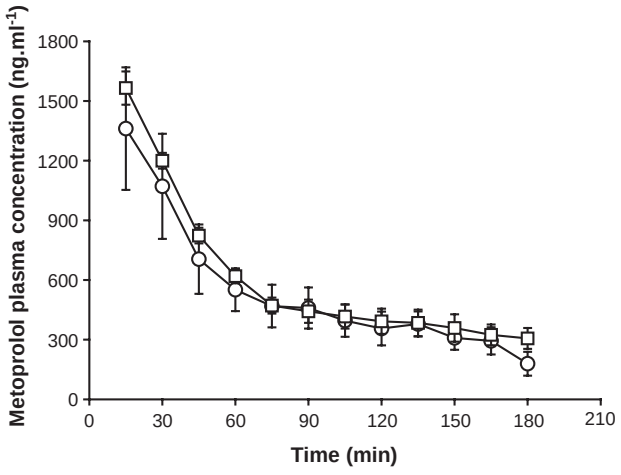

Fig. 1. Mean concentration values of metoprolol vs. time in plasma dialysate of sham operated (circles) rats and aortic coarctated (squares) rats after i.v. administration of metoprolol (3 mg kg^{-1}). Each point shows the mean±SEM of five rats.

Table 1
Pharmacokinetic parameters of metoprolol obtained from dialysate samples: A_{UC} (area under the curve), K_e (constant of elimination), Cl (clearance) and V_d (volume of distribution) in sham operated (SO) and aortic coarctated (ACo) rats after the administration of drug (3 mg kg^{-1} i.v.)

Pharmacokinetic parameter	SO rats	ACo rats
A_{UC} (ng ml^{-1} h^{-1})	1770±696	1603±203
K_e (h^{-1})	1.23±0.28	0.91±0.12
Cl (ml min^{-1})	47.0±5.7	32.8±3.5
V_d (l)	2.3±0.3	2.2±0.1

The data were expressed as mean±SEM of five animals.

anesthetized sham operated (SO) animals and 110±4 mmHg (p<0.05 vs. SO rats) and 391±10 bpm (n=10) in anesthetized aortic coarctated (ACo) rats.

3.1. Pharmacokinetics of metoprolol

Fig. 1 shows the metoprolol concentration–time profile obtained in SO (n=5) and ACo rats (n=5) using the corrected microdialysis data following i.v. administration (3 mg kg^{-1}). The resulting pharmacokinetic parameters are shown in Table 1. No differences between SO and ACo rats were found in the kinetic parameters of metoprolol, such as the area under the curve (A_{UC}), clearance (Cl), volume of distribution (V_d) and constant of elimination (K_e).

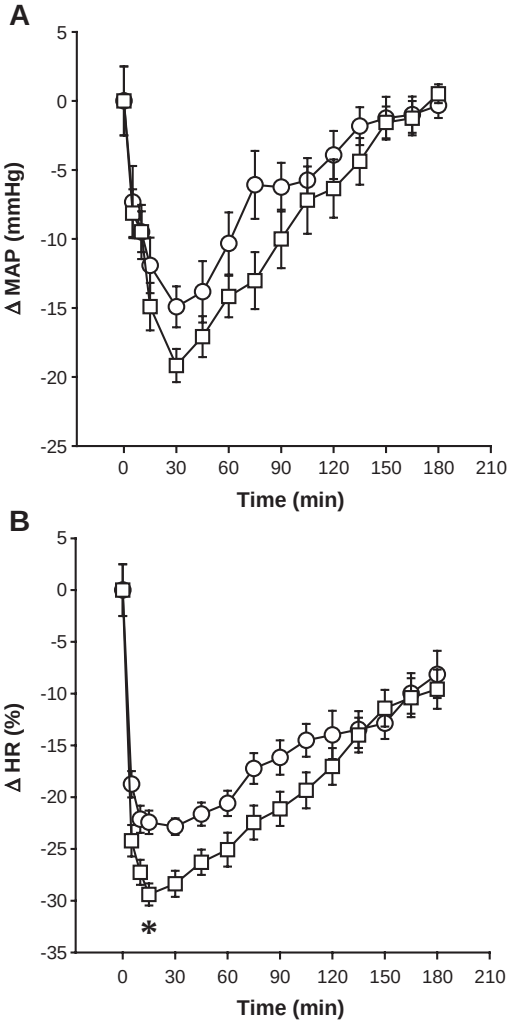

Fig. 2. Time course of the change of mean arterial pressure (ΔMAP)(A) and heart rate (B), after i.v. administration of metoprolol (3 mg kg^{-1}) in sham operated (circles) and aortic coarctated (squares) rats. Each point shows the mean±SEM of five animals. $^{*}p$<0.05 vs. sham operated rats.

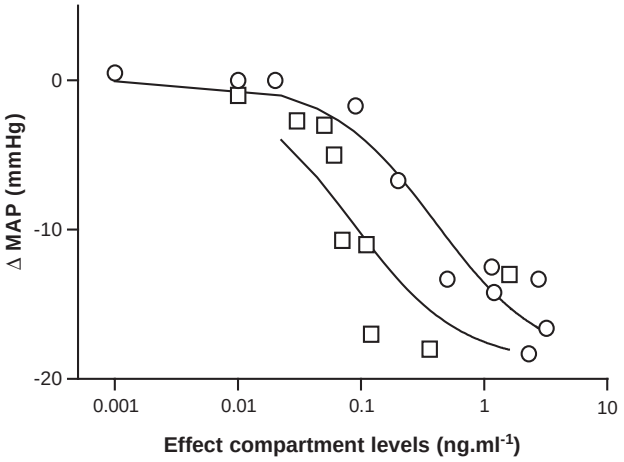

Fig. 3. Change of blood pressure vs. metoprolol effect site concentrations of a representative SO (circles) rat and ACo (squares) animal.

3.2. PK–PD modeling of metoprolol hypotensive effect

Fig. 2A shows the temporal profile of MAP changes following metoprolol i.v. administration (3 mg kg^{-1}) in SO and ACo rats. Metoprolol reduced MAP in both SO and ACo rats without statistical differences between the two experimental groups.

An association between metoprolol concentrations in the effect compartment and the corresponding hypotensive effect was found in both experimental groups. Fig. 3 shows the relationship between the hypotensive effect of metoprolol and its effect site concentrations in representative animals from the SO and ACo groups. No differences were found in the PK–PD parameters calculated in SO and ACo rats (Table 2). The equilibration half-time was also similar between both experimental groups (Table 2). In all cases, a

Table 2
Resulting pharmacokinetic–pharmacodynamic parameters of metoprolol for its hypotensive effect obtained from SO and ACo rats

Pharmacokinetic–pharmacodynamic parameter	SO rats	ACo rats
IC_{50} (ng ml^{-1})	130±60	73±11
E_{max} (%)	−16±2	−20±2
$t_{1/2\ eq}$ (min)	7.3±2.5	7.1±1.3
r^2	0.939	0.950

IC_{50}: metoprolol concentration corresponding to half maximal effect, E_{max}: maximal response, $t_{1/2\ eq}$: equilibration half time between the plasma and the effect compartment, r^2: correlation.
Data were expressed as mean±SEM of five animals.

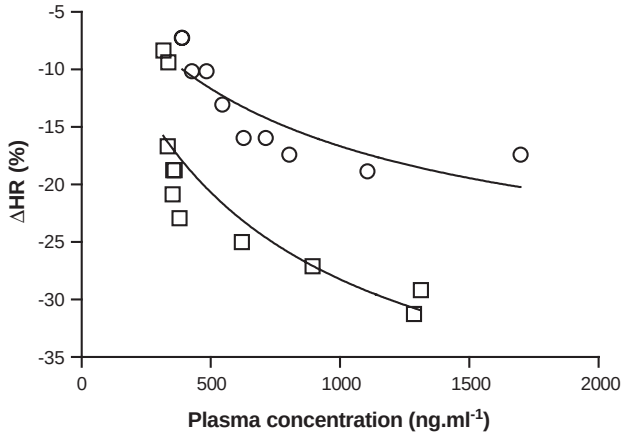

Fig. 4. Change of heart rate vs. metoprolol plasma unbound concentrations of a representative SO (circles) rat and ACo (squares) animal.

good correlation between the concentration of metoprolol in the effect compartment and the pharmacodynamic data was found (Table 2).

3.3. PK–PD modeling of metoprolol chronotropic effect

Fig. 3B shows the temporal profile of heart rate (HR) changes in SO and ACo rats following metoprolol i.v. administration (3 mg kg^{-1}). Metoprolol caused an immediate bradycardia in both experimental groups, but the chronotropic effect was greater in ACo hypertensive rats (ΔHR:−29.4±1.1%; n=5; p<0.05 vs. SO rats) than in SO normotensive rats (ΔFC:−22.4±1.1%; n=5). A correlation between plasma metoprolol concentration and the corresponding chronotropic effect was found in both experimental groups. Fig. 4 shows the relationship between the chronotropic effect of metoprolol and its plasma concentration in representative animals from the SO and ACo groups. As shown in Table 3, the data fitted well to the PK–PD model employed in both experimental groups. Metoprolol exerted a greater chronotropic effect in ACo rats than in SO animals (Table 3). In addition, initial sensitivity to the chronotropic effect of metoprolol was greater in ACo rats than in normotensive rats.

4. Discussion

In the present work, we evaluated the utility of the microdialysis technique for the design of pharmacokinetic–pharmacodynamic (PK–PD) models for antihypertensive drugs. For this purpose, the relationship between metoprolol plasma level and its cardiovascular effect was determined in normotensive sham operated (SO) animals and aortic coarctated (ACo) rats at an early hypertensive stage.

Two different approaches exist to characterise the dose (concentration)–effect relationship, namely the classical dose–response study and pharmacokinetic–pharmacodynamic (PK–PD) modeling (Toutain, 2002). The former method has several disadvantages compared to PK–PD modeling. Dose–response approaches do not provide mechanisms for linking dose and response. On the contrary, PK–PD modeling firstly transforms drug doses into concentrations (the pharmacokinetic process) and then describes the relationship between drug concentration and drug effect (the pharmacodynamic process) (Holford & Sheiner, 1981). This technique therefore allows the determination of the pharmacodynamic parameters EC_{50} and E_{max}. On the other hand, ED_{50} is determined in dose–response trials. This parameter is a hybrid pharmacokinetic and pharmacodynamic parameter, because it combines the EC_{50} value with drug clearance and bioavailability parameters. Another disadvantage of the dose–response studies compared to PK–PD modeling is the need for a greater number of animals. At least 3 dose administrations are required in dose–response studies, whereas in PK–PD modeling the parameters can be estimated following a single dose administration.

One disadvantage of the PK–PD modeling is the need for drug tissue concentration and corresponding pharmacological effect data 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 & 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., 2000). The inlet and one

Table 3
Resulting pharmacokinetic–pharmacodynamic parameters of metoprolol for its chronotropic effect obtained from SO and ACo rats

Pharmacokinetic–pharmacodynamic parameter	SO rats	ACo rats
E_{max} (%)	−26.7±0.9	−37.7±2.9*
S_0 (% μg^{-1} ml)	151±32	68±7*
r^2	0.726	0.715

E_{max}: maximal chronotropic response; S_0 is the initial sensitivity to the chronotropic effect of metoprolol; r^2: correlation.
Data were expressed as mean±SEM of five animals.
* p<0,05 vs. SO rats.

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.

Another disadvantage of PK–PD modeling is the need to cover the complete pharmacodynamic range of a drug after a single administration (Toutain, 2002). Often in clinical pharmacology, it is not possible to determine the maximal effect of a drug by this means, in which case an alternative PK–PD modeling must be applied (Schoemaker et al., 1998). According to previous studies (Höcht, DiVerniero, Opezzo & Taira, 2004), the maximal hypotensive effect of metoprolol is achieved at a dose of 3 mg kg^{-1} exerts. On the other hand, considering that metoprolol exerts a negative chronotropic effect in vitro (Höcht, DiVerniero, Opezzo, & Taira, 2003) and that the maximal effect in vitro is achieved at greater concentrations than the plasma metoprolol concentrations obtained following its systemic administration, it is expected that the intravenous administration of higher doses of metoprolol will produce a further reduction of heart rate. For this reason, an alternative PK–PD model designed by Schoemaker et al. (1998) was applied for PK–PD modeling of the chronotropic effect of metoprolol. This method provides a solution by introducing a new parameter (S_0) equal to E_{max}/EC_{50} that can be used to characterise potency adequately even if there are no signs of a clear maximum (Schoemaker et al., 1998).

The utility of the microdialysis technique for pharmacokinetic–pharmacodynamic modeling has been demonstrated for centrally acting drugs (Chenel et al., 2004) and neuromuscular blockers (Ezzine & Varin, 2005).

A poor concentration–response relationship appears to exist with regard to the hypotensive effects of metoprolol (Myers & Thiessen, 1980; Sklar et al., 1982; von Bahr et al., 1976) although some investigators have found a good association (Esler et al., 1977; Leonetti et al., 1975). Factors that could hamper this poor correlation have been restricted to concentration ranges, high variability and the use of dose, rather than concentration, data (Brynne, Karlsson, & Paalzow, 1998). In the present study, the correlation between the metoprolol concentration and its hypotensive effect was determined. For this purpose, we used a PK–PD model with a separate effect compartment, because of a time delay between metoprolol plasma concentration and the corresponding hypotensive effect (Holford & Sheiner, 1981). In our study, application of the microdialysis technique allowed us to evaluate the hypotensive effect of a wide range of metoprolol concentrations in each animal and to find a good PK–PD correlation. 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 ACo rats. The pharmacodynamic parameters IC_{50} and E_{max} were similar between SO and ACo rats, indicating that the early hypertensive stage did not modify the hypotensive effect of metoprolol.

In the present study, the correlation between metoprolol plasma levels and the corresponding chronotropic effect was also studied. As discussed previously, a modified PK–PD model was applied for the study of the chronotropic effect of metoprolol, because of the impossibility of reaching the maximal chronotropic effect with a single administration of the drug. The data fitted well to the applied PK–PD model, indicating the utility of the method designed by Schoemaker et al. (1998) for the study of pharmacodynamic parameters without attaining the maximal effect. A good correlation was found between metoprolol plasma concentrations and the corresponding bradycardic effect in both experimental groups. Metoprolol exerted a greater maximal effect in ACo rats compared to normotensive animals. Moreover, sensitivity to the chronotropic effect of metoprolol was greater in hypertensive animals than in SO rats. These results suggested that aortic coarctation modified the chronotropic properties of metoprolol, indicating a possible overactivity of the cardiac sympathetic nervous system in this experimental group.

In conclusion, the present work demonstrated the utility of the microdialysis technique for the PK–PD modeling of cardiovascular drugs. The vascular "shunt" microdialysis probe designed in our laboratory allows the continuous and simultaneous determination of metoprolol unbound plasma levels and their corresponding effects on blood pressure and heart rate in the same animal, making the probe suitable for PK–PD studies. A good correlation was found between metoprolol effect compartment concentrations and the corresponding hypotensive effect in normotensive and hypertensive animals. The calculated PK–PD parameters for the antihypertensive effect were not different between the two experimental groups, indicating the absence of a compromised β_1-adrenergic response in the early hypertensive stage of ACo rats. On the other hand, in the present work we also found a good association between metoprolol plasma levels and the corresponding chronotropic effects in SO and ACo rats. The hypertensive rats exhibited a greater sensitivity to the chronotropic effect of metoprolol, suggesting an overactivity of the cardiac sympathetic system in ACo animals at an early hypertensive stage. In this way, the PK–PD modeling not only allows a better pharmacodynamic characterization of metoprolol, but also permits the study of the physiopathological mechanisms of the ACo at an early hypertensive stage.

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References

Ben-hun, J., Cooper, R. L., Cringle, S. J., & Constable, I. J. (1988). Ocular dialysis. A new technique for in vivo intraocular pharmacokinetic measurement. *Archives of Ophthalmology*, *106*, 254–259.

Benveniste, H., & Huttemeier, P. C. (1990). Microdialysis—Theory and application. *Progress in Neurobiology*, *33*, 195–215.

Brodie, M. S., Lee, K., Fredholm, B. B., Ståhle, L., & Dunwiddie, T. V. (1987). Central versus peripheral mediation of responses to adenosine receptor agonist: Evidence against a central mode of action. *Brain Research*, *415*, 323–330.

Brynne, L., Karlsson, M. O., & Paalzow, L. K. (1998). Concentration–effect relationship of L-propranolol and metoprolol in spontaneous hypertensive rats after exercise-induced tachycardia. *Journal of Pharmacological and Experimental Therapeutics*, *286*, 1152–1158.

Chen, Z., & Steger, R. W. (1993). Plasma microdialysis. A technique for continuous plasma sampling in freely moving rats. *Journal of Pharmacological and Toxicological Methods*, *29*, 111–118.

Chenel, M., Marchand, S., Dupuis, A., Lamarche, I., Paquereau, J., Pariat, C., et al. (2004). Simultaneous central nervous system distribution and pharmacokinetic–pharmacodynamic modeling of the electroencephalogram effect of norfloxacin administered at a convulsant dose in rats. *British Journal of Pharmacology*, *142*, 323–330.

Conway, F. J., Fitzgerald, J. D., Mcainsh, J., Rowlands, D. J., & Simpson, W. T. (1976). Human pharmacokinetic and pharmacodynamic studies of atenolol, a new cardioselective beta-adrenoceptor blocking drug. *British Journal of Clinical Pharmacology*, *42*, 665P–666P.

Elmquist, W. F., & Sawchuk, R. J. (1997). Application of microdialysis in pharmacokinetic studies. *Pharmaceutical Research*, *14*, 267–287.

Esler, M., Zweifler, A., Randall, O., & DeQuattro, V. (1977). Pathophysiologic and pharmacokinetic determinants of the antihypertensive response to propranolol. *Clinical and Pharmacological Therapeutics*, *22*, 299–308.

Ezzine, S., & Varin, F. (2005). Interstitial muscle concentrations of rocuronium under steady-state conditions in anaesthetized dogs: Actual versus predicted values. *British Journal of Anaesthesia*, *94*, 49–56.

Gibaldi, M., & Perrier, D. (1982). *Pharmacokinetics* (2nd ed.). New York: Marcel Dekker Inc.

Höcht, C., DiVerniero, C., Opezzo, J. A. W., & Taira, C. A. (2003). In vivo and in vitro pharmacodynamic properties of metoprolol in aortic coarctated rats. *Pharmacological Research*, *47*, 181–188.

Höcht, C., DiVerniero, C., Opezzo, J. A. W., & Taira, C. A. (2004). Pharmacokinetic–pharmacodynamic properties of metoprolol in chronic aortic coarctated rats. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *370*, 1–8.

Höcht, C., Opezzo, J. A. W., & Taira, C. A. (2003). Validation of a new intraarterial microdialysis shunt probe for the estimation of pharmacokinetic parameters. *Journal of Pharmaceutical and Biomedical Analysis*, *31*, 1105–1113.

Holford, N. H. G., & Sheiner, L. B. (1981). Understanding the dose–effect relationship: Clinical application of pharmacokinetic–pharmacodynamic models. *Clinical Pharmacokinetics*, *6*, 429–453.

Hurd, Y. L., Kehr, J., & Ungerstedt, U. (1988). In vivo microdialysis as a technique to monitor drug transport: Correlation of extracellular cocaine levels and dopamine overflow in rat brain. *Journal of Neurochemistry*, *51*, 1314–1316.

Kendall, M. J., Brown, D., & Grieve, A. (1977). Pharmacokinetic and pharmacodynamic studies of a single oral doses of metoprolol in normal volunteers. *European Journal of Drug Metabolism and Pharmacokinetics*, *2*, 73–80.

Leonetti, G., Mayer, G., Morganti, A., Terzoli, L., Zanchetti, A., Bianchetti, G., et al. (1975). Hypotensive and renin-suppressing activities of propranolol in hypertensive patients. *Clinical Science and Molecular Medicine. Supplement*, *48*, 491–499.

Myers, M. G., & Thiessen, J. J. (1980). Metoprolol kinetics and dose response in hypertensive patients. *Clinical and Pharmacological Therapeutics*, *27*, 756–762.

Opezzo, J. A. W., Höcht, C., Taira, C. A., & Bramuglia, G. F. (2000). Study of the evolution of blood and striatal levels of methyldopa: A microdialysis study in sinaortic denervated rat. *Pharmacological Research*, *41*, 455–459.

Rojo-Ortega, J. M., & Genest, J. (1968). A method for production of experimental hypertension in rats. *Canadian Journal of Physiology and Pharmacology*, *46*, 883–885.

Schoemaker, R. C., van Gerven, J. M. A., & Cohen, A. F. (1998). Estimating potency for the Emax-model without attaining maximal effects. *Journal of Pharmacokinetics and Biopharmaceutics*, *26*, 581–593.

Sklar, J., Johnston, D., Overlie, P., Gerber, J. G., Brammell, H. L., Gal, J., et al. (1982). The effects of a cardioselective (metoprolol) and a nonselective (propranolol) beta-adrenergic blocker on the response to dynamic exercise in normal men. *Circulation*, *65*, 894–899.

Toutain, P. L. (2002). Pharmacokinetic/Pharmacodynamic integration in drug development and dosage–regimen optimization for veterinary medicine. *AAPS Pharmaceutical Sciences*, *4*, 1–29.

Ungerstedt, U. (1991). Microdialysis—Principles and applications for studies in animals and man. *Journal of Internal Medicine*, *230*, 365–373.

von Bahr, C., Collste, P., Frisk-Holmberg, M., Haglund, K., Orme, M., Ostman, J., et al. (1976). Plasma levels and effects of metoprolol on blood pressure, adrenergic beta receptor blockade and plasma renin activity in essential hypertension. *Clinical and Pharmacological Therapeutics*, *20*, 130–137.

Zacest, R., & Koch-Weser, J. (1972). Relation of propranolol plasma levels to b-blockade during oral therapy. *Pharmacology*, *7*, 178–184.