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Research Paper

Increased sensitivity to diltiazem hypotensive effect in an experimental model of high-renin hypertension

Facundo M. Bertera[a], Marcos A. Mayer[a,b], Javier A.W. Opezzo[a,b], Carlos A. Taira[a,b] and Christian Höcht[a,b]

[a]Cátedra de Farmacología and [b]Instituto de Fisiopatología y Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, (C1113AAD) Buenos Aires, Argentina

Abstract

Objectives The aim of this work was to evaluate the pharmacokinetic–pharmacodynamic properties of diltiazem in an experimental model of high-renin hypertension, such as the aortic coarctated (ACo) rat, to further characterize the responsiveness of this model to calcium channel blockers.

Methods A 'shunt' microdialysis probe was inserted in a carotid artery of anaesthetized ACo and control sham-operated (SO) rats for simultaneous determination of diltiazem plasma concentrations and their effects on mean arterial pressure and heart rate after the intravenous application of 3 and 6 mg/kg of the drug. Correlation between plasma levels and cardiovascular effects was established by fitting the data to a modified E_{max} model.

Key findings Volume of distribution was greater in ACo than in SO rats. Diltiazem plasma clearance (Cl) was significantly greater in ACo rats than in normotensive SO rats after administration of diltiazem (6 mg/kg). Moreover, Cl increased with dose in ACo but not in SO rats. No differences were observed in the maximal bradycardic effect comparing both experimental groups, and sensitivity (S_0) to diltiazem chronotropic effect was similar comparing SO and ACo rats. Differences were not found in the maximal response of the hypotensive effect comparing SO and ACo rats, but the S_0 to diltiazem hypotensive effect was greater in ACo rats than in SO rats.

Conclusions ACo induced profound changes in diltiazem pharmacokinetic behaviour. In addition, our results suggested an increased sensitivity to diltiazem blood pressure lowering effect in experimental renovascular hypertension with high-renin levels.

Keywords aortic coarctation rats; diltiazem; hypotensive effect; microdialysis; pharmacokinetic–pharmacodynamic modelling

Introduction

Calcium channel blockers, such as diltiazem, interfere with L-calcium channels in the vascular smooth muscle and at the myocardium,[1] exerting an antihypertensive effect.[1] Although calcium channel blockers have been long used as first-line therapy of hypertension, their role in high-renin hypertension remains controversial. Early reports suggest that these antihypertensive agents might not be effective in the treatment of high-renin hypertension,[2,3] considering that antihypertensive efficacy of calcium channel blockers inversely correlates with plasma renin levels. However, Preston *et al.*[4] have demonstrated that diltiazem exerts a consistent antihypertensive response regardless of renin profile. In addition, these drugs have been found to be effective in renovascular hypertension, a secondary form of hypertension characterized by high plasma renin activity.[5]

Pharmacokinetic–pharmacodynamic (PK-PD) properties of calcium channel blockers have been scarcely studied in animal models of hypertension. Considering the controversial role of calcium channel blockers in high-renin hypertension, it is of interest to describe the antihypertensive efficacy of this therapeutic group in an animal model of renovascular hypertension, such as aortic coarctated rats. The hypertensive state produced by coarctation of the abdominal aorta in rats is attributed mainly to activation of the renin–angiotensin system, vasopressin and changes in sodium homoeostasis.[6–9]

Correspondence: Dr Christian Höcht, Cátedra de Farmacología, Instituto de Fisiopatología y Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, (C1113AAD) Buenos Aires, Argentina.
E-mail: chocht@ffyb.uba.ar

PK-PD modelling of cardiovascular effects of antihypertensive drugs is a powerful tool to evaluate the pathophysiology of the hypertensive stage in different experimental models.[10] PK-PD modelling not only allows better pharmacodynamic characterization of drugs but also permits screening and dosage-regimen selection.[11] PK-PD modelling also allows the identification of biomarkers and animal models for efficacy and toxicity and the exploration of any dissociation between plasma concentration and duration and onset of pharmacological effect.[12]

In accordance, the aim of this work was to evaluate the PK-PD properties of diltiazem in an experimental model of high-renin hypertension, such as the aortic coarctated rat, to further characterize the responsiveness of this kind of hypertension to calcium channel blockers.

Materials and Methods

Induction of hypertension

Male Sprague-Dawley rats were used, 250–270 g. Animal experiments were performed in accordance with the *Principles of laboratory animal care* (NIH publication No. 85-3, revised 1985). The animal experiments were approved by the local Scientific and Technology Ethics Committee at the University of Buenos Aires. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Abdominal aortic coarctation (ACo) was carried out according to Rojo-Ortega and Genest[13] in rats anaesthetized with ether. Briefly, the technique consisted of banding the aorta between the two renal arteries. Control rats were sham-operated (SO). Experiments were carried out five days after surgery.

Experimental design

Experiments were performed on animals anaesthetized with a mixture of chloralose (50 mg/kg, i.p.) and urethane (500 mg/kg, i.p.). A femoral vein was cannulated for the intravenous administration of isotonic solution containing diltiazem at a dose of 3 and 6 mg/kg. A validated 'shunt' microdialysis probe with one vascular inlet and two vascular outlets[14] was used for examining the time course of free diltiazem plasma concentrations. The inlet and vascular outlet of the heparinized probe (50 U/ml) were inserted into the left carotid artery, while the remaining vascular outlet was connected to a Spectramed P23XL pressure transducer (Spectramed, Oxnard, CA, US) coupled to a Grass 79D polygraph (Grass Instrument, Quincy, MA, US). 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.

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 (Bee Hire, BAS, West Lafayette, IN, US). The flow rate was 2 μl/min and samples were collected at 15-min intervals.

After placement of the microdialysis probe, the in-vivo recovery of diltiazem was evaluated in all experiments using reverse microdialysis[15] by perfusing the microdialysis probe with a solution of diltiazem (2 μg/ml) and by taking the proportion lost across the dialysis membrane as an estimate of the recovery.

The in-vivo recovery of diltiazem was calculated using Equation 1:

$$R = (C_{in} - C_{out})/C_{in} \quad (1)$$

where R is the diltiazem in-vivo recovery, C_{in} is the concentration of diltiazem in the perfusate and C_{out} is the concentration of diltiazem in the dialysate. Recovery of diltiazem in all experiments was 0.26 ± 0.06.

After determination of the in-vivo recovery, basal values of MAP and HR were determined during a 30-min interval, followed by intravenous administration of 3 and 6 mg/kg of diltiazem. For drug administration, (+)-*cis*-diltiazem (Sigma, St Louis, MO, US) was dissolved in Ringer solution and administered during 2 min. MAP and HR were monitored for 2 h after drug administration with the simultaneous recollection of microdialysate samples every 5 min during the first 20-min interval and every 15 min afterwards. Rats were under anaesthesia during the entire experiment. The anaesthetic state was evaluated by the determination of the palpebral reflex and supplements of anaesthesia were administered if necessary.

Analytical determination of diltiazem in dialysate samples

Diltiazem dialysate levels were determined by high-performance liquid chromatography (HPLC) with ultraviolet detection. Dialysate samples were injected without pretreatment into a chromatographic system equipped with a Phenomenex Luna 5 μm, C18, 250 mm × 4.60 mm column (Phenomenex, Torrance, CA, US) and an ultraviolet detector (UVIS 204; Linear Instruments, Reno, NV, US). The mobile phase composed of 0.05 M potassium dihydrogen phosphate buffer–acetonitrile–triethylamine (65 : 35 : 0.2; pH 3.0 with phosphoric acid) was pumped at a flow rate of 1.4 ml/min. Column effluents were detected at a wavelength of 237 nm. Linearity of the analytical method was determined in the range of 20–5000 ng/ml. Limit of quantification of our HPLC method was 5 ng/ml.

Analysis of data

Correction of microdialysis data

To determine blood-unbound diltiazem levels from the microdialysis data, drug concentrations in the microdialysis samples were adjusted using the in-vivo probe recovery. So, the unbound diltiazem concentrations in blood (C) were calculated using Equation 2:

$$C_u = C_{out}/R \quad (2)$$

where C_u is the calculated unbound diltiazem concentration, C_{out} is diltiazem concentration in the dialysate and R is the in-vivo recovery of the microdialysis probe.

On the other hand, microdialysis generates data that are the integral of the concentration surrounding the probe during the sampling interval.[16] Therefore, the microdialysis data must be transformed from a series of integrals to a series of points corresponding to the end time of the sample interval, as previously reported.[17]

Diltiazem pharmacokinetics

Compartment analysis of diltiazem pharmacokinetics was used. The temporal profile of diltiazem concentration obtained from the corrected microdialysis data following bolus dosing was described by a two-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) that uses a cyclic three-stage optimization routine (one-dimensional direct search; vectorial direct search/Hooke-Jeeves modified; Gauss-Newton/Marquadt modified). The area under the curve (AUC) of diltiazem levels versus time (from 0 to infinity) was calculated using the trapezoidal rule. Clearance (Cl) and steady-state volume of distribution (Vdss) were calculated by standard methods.[18]

PK-PD modelling of diltiazem cardiovascular effect

In the diltiazem PK-PD relationship study, the diltiazem plasma concentration and changes in MAP and HR were used. Considering that no time delay between the plasma concentrations of diltiazem and their cardiovascular response was observed, unbound plasma concentrations were directly linked to the pharmacological response.[19] The PK-PD model used to simulate cardiovascular effects of diltiazem was based on a two-compartment pharmacokinetic model with the pharmacological response directly linked from the central compartment.

As described previously, in our experimental protocol, the application of a high dose of diltiazem induced sinus arrest in both spontaneously hypertensive and Wistar Kyoto rats.[17] Therefore, it is impossible to reach maximal response for the bradycardic and hypotensive effect of diltiazem. To apply in this kind of experimental conditions, an alternative PK-PD model for data analysis was designed by Schoemaker *et al.*[20] The authors replaced the concentration yielding half-maximal response (EC50) with S_0 in the E_{max} equation. S_0 is the slope of the tangent at concentration zero and represents the initial sensitivity to the drug. This parameter is dose independent, taking into consideration that the initial part of the E_{max} curve does not change with increasing concentrations and the tangent to the concentration–effect curve at zero concentration does not depend on the rest of the curve.[20] In previous work, we found that the modified E_{max} model allows an accurate estimation of diltiazem sensitivity in conditions when maximal pharmacological response cannot be attained.[17]

PK-PD modelling of diltiazem chronotropic and blood-lowering effect was carried out using the ADAPT II software package[21] by applying the maximum likelihood method as estimation procedure of PK-PD parameters. Data were adjusted to Equation 3:

$$E = S_0 \times E_{max} \times C(t)/(E_{max} + (S_0 \times C(t))) \quad (3)$$

where E is the drop in HR and MAP, S_0 is the initial sensitivity to diltiazem, E_{max} is the maximal response, and C(t) is the diltiazem plasma concentration at time t. As relative response was used for PK-PD simulations, E_{max} was constrained to be less than or equal to 100%.

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 unpaired Student's *t*-test or by two-way analysis of variance and the test of Bonferroni as a post-hoc test. Pharmacokinetic and PK-PD parameters were log transformed for statistical analysis to reduce heterogeneity of the variance. Goodness of fit of pharmacokinetic and PK-PD simulations was established by the Akaike information criterion (AIC). Statistical analysis of pharmacokinetic and PK-PD parameters was performed by a two way analysis of variance and the test of Bonferroni as the post hoc-test. The correlation between diltiazem maximal hypotensive effect and basal blood pressure values of SO and ACo rats was studied by means of Pearson's test.

Statistical tests were performed by using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). Statistical significance was defined as $P < 0.05$.

Results

Basal values of MAP and HR were 90.6 ± 2.8 mmHg and 409 ± 9 beats/min (n = 14), respectively, in anaesthetized SO rats and 106.8 ± 2.5 mmHg ($P < 0.05$ vs SO rats) and 438 ± 7 beats/min ($P < 0.05$ v SO rats) (n = 14), respectively, in anaesthetized ACo rats.

Pharmacokinetics of diltiazem

Figure 1 shows the diltiazem concentration–time profile obtained from the microdialysis corrected data from SO rats (n = 7) and ACo rats (n = 7) after intravenous administration of 3 and 6 mg/kg of the drug. A biexponential decay of plasma diltiazem levels was found in all experiments compatible with a pharmacokinetic two-compartment model. Moreover, data fitted better to a two-compartment model (AIC = 68.7) than a mono-compartment pharmacokinetic model (AIC = 88.1). At both dose levels, plasma concentrations of diltiazem were lower in ACo rats than in SO rats (Figure 1). The resulting pharmacokinetic parameters are shown in Table 1. C_{max} of

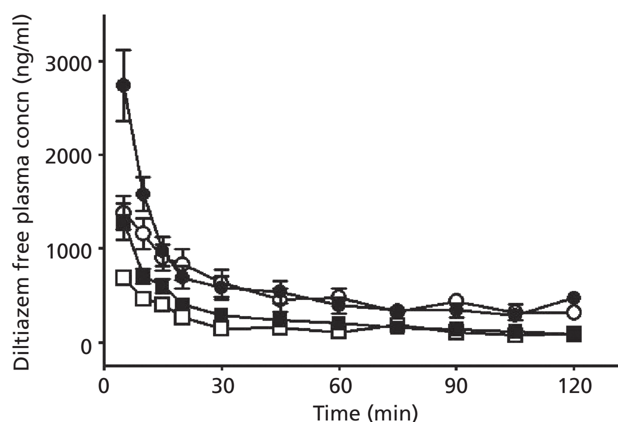

Figure 1 Pharmacokinetic profile of diltiazem. Mean concentration values of diltiazem vs time in plasma dialysate of sham-operated (SO) rats (circles) and aortic coarctated (ACo) rats (squares) after intravenous administration of diltiazem (3 mg/kg, white symbols; 6 mg/kg, black symbols). Each point shows the mean ± SEM of seven rats.

Table 1 Pharmacokinetic parameters of diltiazem obtained from dialysate samples

Pharmacokinetic parameter	SO rats		ACo rats	
	3 mg/kg	6 mg/kg	3 mg/kg	6 mg/kg
α (1/h)	7.1 ± 0.7	10.7 ± 1.7	9.6 ± 1.5	10.9 ± 1.0
β (1/h)	0.69 ± 0.13	0.57 ± 0.18	0.75 ± 0.29	0.69 ± 0.13
Cl (ml/min)	39 ± 8	45 ± 13	63 ± 12	110 ± 13*#
V_{dss} (L)	3.6 ± 0.8	4.6 ± 0.8	6.7 ± 0.8*	8.1 ± 1.0*
AUC (ng/ml h)	1546 ± 241	3171 ± 504#	633 ± 110*	1009 ± 115*#
C_{max} (ng/ml)	1816 ± 222	4286 ± 288#	1035 ± 114*	2194 ± 325*#

SO, sham operated; ACo, aortic coarctated; α constant of distribution; β, constant of elimination; Cl, clearance; V_{dss}, volume of distribution at the steady state; AUC, area under the curve; C_{max}, maximal plasma concentration extrapolated at time 0. Parameters were determined in SO and ACo rats after the intravenous administration of drug (3 mg/kg and 6 mg/kg). *$P < 0.05$ vs SO rats; #$P < 0.05$ vs 3 mg/kg diltiazem.

diltiazem was smaller in ACo rats than in SO rats after the administration of 3 and 6 mg/kg of the drug. Consequently, V_{dss} was greater in ACo rats than in SO rats at both dose levels (Table 1). Diltiazem plasma Cl was significantly greater in ACo rats than in normotensive SO rats after administration of 6 mg/kg of diltiazem. In addition, Cl increased with dose in ACo rats but not in normotensive SO rats. A proportional increase of AUC and C_{max} with dose increment was observed in both experimental groups.

PK-PD modelling of the chronotropic effect of diltiazem

Figure 2 shows the time course of HR after intravenous administration of diltiazem in SO and ACo rats. Change of HR was expressed as percentage of basal value during 30 min before administration of the drug. A dose-dependent increase of the bradycardic effect of diltiazem was observed in SO rats (3 mg/kg: ΔHR –31.9 ± 2.5%, $n = 7$; 6 mg/kg; ΔHR –46.9 ± 2.6%, $n = 7$) and ACo rats (3 mg/kg: ΔHR –27.2 ± 4.5%, $n = 7$; 6 mg/kg: ΔHR –45.6 ± 2.6%, $n = 7$) (Figure 2). Although maximal chronotropic effect of diltiazem was similar comparing both experimental groups,

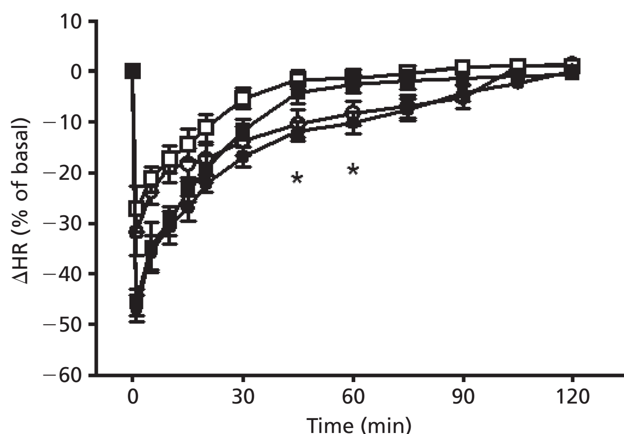

Figure 2 Chronotropic response to intravenous administration of diltiazem in rats. Time course of heart rate changes (ΔHR) in sham-operated (SO) rats (circles) and aortic coarctated (ACo) (squares) after intravenous administration of diltiazem (3 mg/kg, white symbols; 6 mg/kg, black symbols). Each point shows the mean ± SEM of seven rats. *$P < 0.05$ vs SO rats.

HR returned to baseline values earlier in ACo rats than in SO rats at both dose levels (Figure 2).

Table 2 shows PK-PD parameters estimation for the chronotropic effect of diltiazem using the modified E_{max} pharmacodynamic model. No differences were observed in the maximal chronotropic effect comparing both experimental groups. Sensitivity to diltiazem chronotropic effect, expressed as S_0, was similar comparing SO and ACo rats (Table 2). Estimation of diltiazem PK-PD parameters for the chronotropic effect was dose independent in both experimental groups (Table 2).

PK-PD modelling of the hypotensive effect of diltiazem

The time courses of MAP after intravenous administration of diltiazem (3 or 6 mg/kg) in SO rats and ACo rats are depicted in Figure 3 (a, b). Change of MAP was expressed as absolute change in MAP (Figure 3a) and as percentage of basal value during the 30 min before administration of the drug (Figure 3b). A dose-dependent increase of the blood lowering effect of diltiazem was observed in both experimental groups. Although diltiazem (6 mg/kg) induced a greater hypotensive effect, expressed as absolute change in ACo rats (ΔMAP –60.8 ± 3.5 mmHg, $n = 7$, $P < 0.05$ vs SO rats) compared with SO rats (ΔMAP –47.5 ± 2.5 mmHg, $n = 7$) (Figure 3a), relative blood pressure effect of diltiazem was not different between SO (3 mg/kg: ΔMAP –59.6 ± 2.8%, $n = 7$; 6 mg/kg: ΔMAP –69.6 ± 2.9%, $n = 7$) and ACo rats (3 mg/kg: ΔMAP –58.6 ± 2.8%, $n = 7$; 6 mg/kg: ΔMAP –75.7 ± 2.4 mmHg, $n = 7$) (Figure 3b).

As shown in Figure 4, a good relationship was found between basal blood pressure values of SO and ACo rats with the maximal absolute change of MAP induced by the administration of 3 mg/kg ($r = 0.7352$; $P < 0.05$) and 6 mg/kg of diltiazem ($r = 0.9037$; $P < 0.05$).

The obtained PK-PD parameters for the hypotensive effect of diltiazem after administration of 3 and 6 mg/kg in SO and ACo rats are shown in Table 2. No differences were found in the maximal response comparing SO and ACo rats (Table 2). Sensitivity to diltiazem hypotensive effect, expressed as S_0, was greater in ACo rats than in SO rats (Table 2). Estimation of diltiazem PK-PD parameters for the hypotensive effect was dose independent in both experimental groups (Table 2).

Table 2 Pharmacokinetic–pharmacodynamic parameter estimation of diltiazem chronotropic and hypotensive effect obtained from sham-operated and aortic coarctated rats by the application of the modified E_{max} model

PK-PD parameter	SO rats		ACo rats	
	3 mg/kg	6 mg/kg	3 mg/kg	6 mg/kg
Chronotropic effect				
E_{max} (%)	81.2 ± 9.1	92.5 ± 7.1	91.7 ± 5.1	92.8 ± 5.8
S_0 (%/μg^{-1} ml)	44.2 ± 7.1	55.2 ± 9.8	41.4 ± 5.4	47.2 ± 11.3
AIC	68.3	75.8	54.5	59.9
Hypotensive effect				
E_{max} (%)	92.4 ± 5.5	93.6 ± 6.2	97.5 ± 3.5	94.9 ± 3.4
S_0 (%/μg^{-1} ml)	86.1 ± 11.3	84.3 ± 14.6	176.5 ± 22.1*	147.8 ± 17.0*
AIC	109.2	88.7	66.2	83.4

SO, sham-operated; ACo, aortic coarctated; E_{max}, maximal response; S_0, initial sensitivity to the chronotropic effect of diltiazem; AIC, Akaike information criterion. Data are expressed as mean ± SEM of seven rats. *$P < 0.05$ vs SO rats.

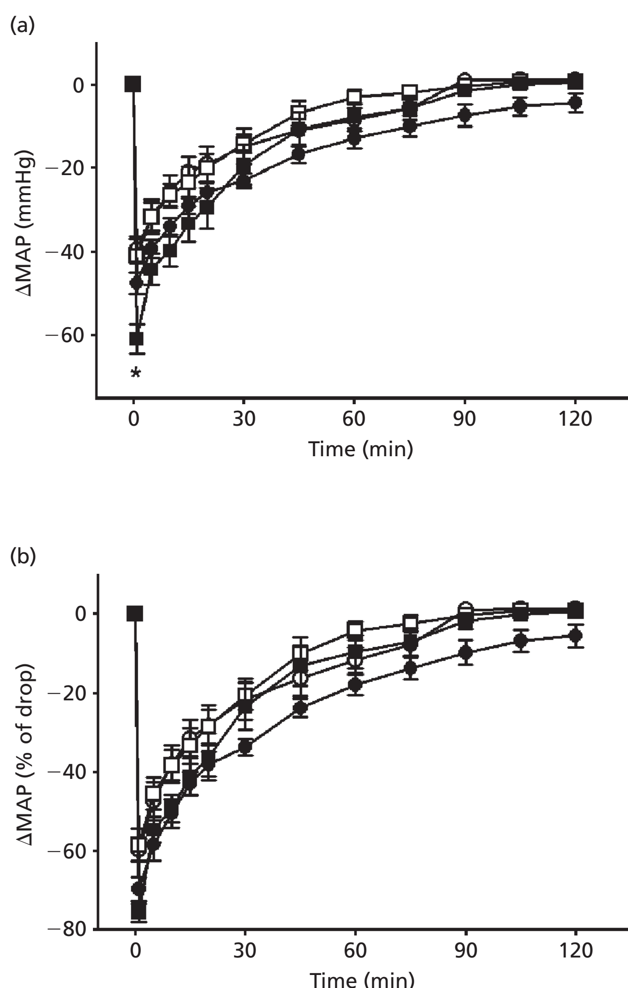

Figure 3 Hypotensive response to intravenous administration of diltiazem in rats. Time course of the change of mean arterial pressure (ΔMAP), expressed as absolute change (a) and relative change to basal values (b) in sham-operated (SO) rats (circles) and aortic coarctated (ACo) rats (squares) after intravenous administration of diltiazem (3 mg/kg, white symbols; 6 mg/kg, black symbols). Each point shows the mean ± SEM of seven rats. *$P < 0.05$ vs SO rats.

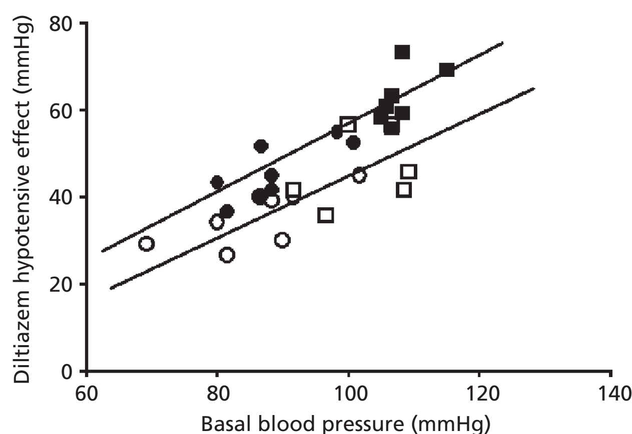

Figure 4 Relationship between diltiazem hypotensive effect and basal blood pressure level in rats. Correlation between maximal absolute hypotensive response to diltiazem and basal blood pressure values of sham-operated (SO) rats (circles) and aortic coarctated rats (squares) after intravenous administration of 3 mg/kg (white symbols) and 6 mg/kg (black symbols) of diltiazem. Correlation analysis was made on pooled data.

Discussion

In this work, PK-PD analysis of diltiazem cardiovascular effects showed that aortic coarctation induced significant changes in the pharmacokinetic and pharmacodynamic behaviour of diltiazem.

Diltiazem pharmacokinetic properties have been extensively studied in both clinical and basic research.[22–24] Diltiazem undergoes extensive hepatic biotransformation mediated by cytochrome P450 (CYP)3A4 isoenzyme showing a hepatic extraction fraction of 0.87 in normotensive rats.[25] In addition, plasma diltiazem is 80% bound to albumin.[26]

Pharmacokinetic properties of diltiazem were evaluated in SO and ACo rats using the microdialysis technique. Microdialysis sampling has been extensively used for the evaluation of plasma concentrations of therapeutic agents in basic research,[27] considering that this technique allows a continuous monitoring of unbound plasma concentrations

of drug without fluid removal. Previously, we have demonstrated the utility of our shunt microdialysis intra-arterial probe for the study of pharmacokinetics of diltiazem.[17]

In addition, it is important to mention that we studied diltiazem pharmacokinetics in urethane–chloralose anaesthetized SO and ACo rats. Meneguz *et al*.[28] and Loch *et al*.[29] have demonstrated that urethane inhibits hepatic biotransformation of ethylmorphine and ethosuximide, two CYP3A substrates, by approximately 40%. However, it is unlikely that urethane interferes with diltiazem pharmacokinetics in our experimental conditions, considering that systemic plasma clearance of diltiazem is high and dependent on liver blood flow. In addition, urethane dose used in the present work is lower than the dosing used by Loch *et al*.[29] and Meneguz *et al*.[28] in their previous reports.

Diltiazem pharmacokinetics have been previously evaluated by us and other authors in spontaneously hypertensive rats (SHR) and normotensive rats.[17,30] In these previous reports, a dose-dependent increment of Cl was found in the hypertensive rats but not in normotensive rats. In our current work, we also found an increase of the estimated Cl with diltiazem dosing in ACo rats but not in SO rats. To explain this finding, it is important to take into account that the hepatic metabolic rate of diltiazem depends on hepatic blood flow; an increase of diltiazem dose could produce a greater hepatic perfusion due to vasodilatation, enhancing drug biotransformation. In accordance, although changes in hepatic blood flow by diltiazem administration were not directly evaluated in this study, several reports have demonstrated enhanced hepatic perfusion due to diltiazem administration.[23,31] Although diltiazem also increases renal blood flow,[32,33] it is improbable that increment in renal blood flow induced by diltiazem modified diltiazem clearance, considering that only traces of diltiazem are eliminated in urine.[22]

We also found an altered pharmacokinetic behaviour in ACo rats compared with normotensive SO rats. Plasma clearance of diltiazem was significantly greater in ACo than in SO rats after administration of 6 mg/kg of the drug but not after administration of the lower dose. Taking into account that diltiazem hepatic biotransformation is flow dependent, our results suggested that diltiazem generates a greater vasodilatation of hepatic vessels in ACo rats than in normotensive SO rats.

In this study, a greater V_{dss} of diltiazem was found in ACo rats than in SO normotensive rats. In a previous study,[17] we also found an enhanced tissue distribution of diltiazem in SHR compared with WKY rats. Although the pathophysiology of the hypertensive state is clearly different comparing both experimental models of hypertension, diltiazem shows similar pharmacokinetic alterations in ACo rats and SHR compared with their corresponding normotensive control group. In the previous study,[17] we postulated that the increased volume of distribution of diltiazem, considering its lipophilicity, could be a consequence of increased adipose tissue of SHR. However, it is improbable that ACo rats present an altered body composition in favour of increased adipose tissue.

To explain the increased diltiazem volume of distribution in ACo rats, it is important to take into account that tissue distribution of a drug depends not only on its lipophilicity, but also on plasma protein binding, efflux transporter activity and tissue protein binding.[34] In this way, diltiazem shows a high affinity to L-type calcium channels and therefore alteration in the expression of this channel could modify tissue distribution of diltiazem.[1] It has been found that the hypertensive state, independently of its pathophysiological basis, induces an increase in calcium-channel expression in different vascular beds.[35] Pesic *et al*.[36] found a 3.25-fold increased expression of the pore-forming α1C subunit of the L-type calcium channel in the right renal artery of ACo rats at two and seven days after banding of abdominal aorta. Taken together, the enhanced volume of distribution of diltiazem observed in ACo rats could be a consequence of an increased binding of the drug to vascular calcium channels.

Although an alternative explanation to the increased volume of distribution of diltiazem in ACo rats could be a lower plasma protein binding of the drug, in a previous report[17] we did not find alterations in diltiazem plasma protein binding in SHR with regard to WKY animals.

PK-PD modelling of the hypotensive and chronotropic effect of diltiazem was also evaluated in ACo and SO rats by means of microdialysis sampling. It is well known that an accurate estimation of PK-PD parameters using the E_{max} model needs determination of the complete pharmacodynamic range after application of a single dose of the drug.[11] When the E_{max} model is used to estimate a curve without a clear maximum, E_{max} and EC50 estimates are extremely variable while their ratio is far less so.[20] Schoemaker *et al*.[20] designed a modified pharmacodynamic model by replacing the parameter E_{max}/EC50 with S_0 in the E_{max} equation. S_0 is a more stable parameter and can be interpreted as the initial sensitivity to the drug at low concentrations. In our experimental conditions, reaching maximal response to the hypotensive and bradycardic effect of diltiazem was impossible, considering that the application of a higher dose of the calcium channel blocker (10 mg/kg) induced sinus arrest in a high proportion of the rats (data not shown).

In a previous report, we found that estimation of diltiazem sensitivity using the modified E_{max} equation allows a more precise and accurate estimation of PK-PD properties of diltiazem. Therefore, we used the modified E_{max} pharmacodynamic model to evaluate PK-PD properties for diltiazem cardiovascular effects.[17]

It is also important to mention that generation of active metabolites of diltiazem, such as deacetyl diltiazem,[37] do not significantly contribute to its pharmacological action[38,39] and, therefore, diltiazem plasma levels could be directly related to their corresponding cardiovascular response.

In this work, diltiazem induced a dose-dependent decrease in HR in both experimental groups without differences in the maximal chronotropic effect between SO and ACo rats. Conversely, HR returned earlier to baseline values in ACo rats than in SO rats. PK-PD analysis of diltiazem bradycardic response did not show alterations in diltiazem sensitivity

comparing ACo rats and SO rats, suggesting that ACo did not affect cardiac response to diltiazem. Considering that maximal bradycardic response is similar between both experimental groups, but the same is attained at lower diltiazem concentrations on ACo rats with regard to SO rats, it could be proposed that sensitivity to diltiazem chronotropic effect is greater in ACo rats than in normotensive rats. However, it is important to take into account that S_0 estimates the initial sensitivity to diltiazem pharmacological response, considering that this parameter represents the slope of the tangent at concentration zero of the E_{max} curve. Therefore, estimation of S_0 did not differ comparing both experimental groups, considering that earlier recovery of HR baseline values in ACo rats is associated with lower levels of the drug in the hypertensive group.

However, the main objective of this work was to establish diltiazem sensitivity to its hypotensive effect to gain information regarding the antihypertensive efficacy of calcium channel blockers in high-renin hypertension. As discussed previously, early reports have suggested that calcium channel blockers are not effective in the treatment of high-renin hypertension, considering that antihypertensive efficacy of calcium channel blockers inversely correlates with plasma renin levels.[2,3] However, controversy exists with regards to their efficacy in renovascular hypertension, a secondary form of hypertension characterized by high plasma renin activity. Although calcium channel blockers have been found to be effective in renovascular hypertension in clinical practice,[5] Zawada & Johnson[40] showed the absence of blood pressure lowering effect of these drugs in experimental renovascular hypertension. Although functionality of calcium channels has been extensively studied in ACo rats, to the best our knowledge, no in-vivo studies have been made to establish the blood pressure lowering efficacy of diltiazem in ACo rats. In-vitro studies have demonstrated that the increased vascular tone of aorta from ACo rats is a consequence of increased cytoplasmatic calcium levels.[41] Moreover, a good correlation between calcium content and elevated blood pressure was found in rats with ACo.[42] In addition, as mentioned above, the hypertensive state induced by aortic coarctation enhanced calcium channel expression in vascular beds.[36]

Although absolute hypotensive response to diltiazem was significantly greater in ACo rats than in normotensive SO rats after administration of 6 mg/kg, no differences were found in the relative hypotensive response comparing both experimental groups. These results are in accordance with the fact that the hypotensive efficacy of calcium channel blockers is related to pretreatment blood pressure level.[43]

Although hypotensive response to diltiazem was not increased in ACo rats compared with SO rats, the same was obtained at significantly lower diltiazem plasma concentrations in the hypertensive group compared with normotensive SO rats. As expected, PK-PD analysis of diltiazem hypotensive response showed enhanced diltiazem sensitivity to its blood pressure lowering effect in hypertensive ACo rats with regard to normotensive SO rats. In addition, maximal hypotensive response to diltiazem was similar comparing both experimental groups. These results are in accordance with the fact that ACo rats showed a greater expression of vascular L-type calcium channels[36] and a corresponding enhanced calcium influx through L-type calcium channels.[44] At this point, it is important to mention that in a previous study we also found an increased sensitivity to diltiazem hypotensive response in SHR. However, in the present study the increase in diltiazem sensitivity was nearly two fold in ACo rats compared with control rats; SHR showed a four-fold increase in drug sensitivity. Greater sensitivity to diltiazem cardiovascular effects in SHR with regards to ACo rats could also be explained by the fact that ACo rats tolerate administration of higher doses of diltiazem. In this work, cardiovascular response to 3 and 6 mg/kg of diltiazem has been assessed in all experiments. Conversely, as shown previously,[17] administration of high doses of diltiazem (e.g. 6 and 10 mg/kg) produced a high mortality rate in SHR due to an excessive cardiovascular response. Therefore, in this previous work,[17] diltiazem PK-PD properties were evaluated in SHR after application of 1 and 3 mg/kg of the drug.

Another point that needs to be discussed is the fact that ACo and SHR show increased sensitivity to diltiazem hypotensive response, although the hypertensive stage in these experimental models is induced and maintained by clearly different pathophysiological mechanisms. Therefore, our results suggest that increased calcium vascular influx is a common final pathway of neurohormonal mechanisms involved in the maintenance of the hypertensive stage in ACo rats and SHR. Considering that SHR and ACo represent different models of experimental hypertension, these differences in the enhanced sensitivity might explain the variability in therapeutic responses observed in different types of hypertension in the clinical setting.

Conclusions

The hypertensive state induced by ACo induced profound alterations in the pharmacokinetic and pharmacodynamic properties of diltiazem. The pharmacokinetic profile of diltiazem in ACo rats showed an increased systemic clearance and volume of distribution, possibly as a consequence of an enhanced vasodilatatory effect of diltiazem and an increased expression of vascular L-type calcium channel, respectively. Moreover, increased influx of calcium in vascular beds of ACo rats also explains the enhanced sensitivity to diltiazem blood pressure lowering effect in ACo rats with regard to the SO group. Our results suggest an increased sensitivity to diltiazem blood pressure lowering effect in experimental renovascular hypertension with high-renin levels.

Finally, estimation of PK-PD parameters of calcium channel blockers by means of PK-PD modelling seems to be a powerful tool to define the role of calcium channel blockers in high-renin hypertension. Previous reports showing the lack of efficacy of calcium channel blockers in high-renin hypertension were based on populational data rather than evaluation of individual subjects.[2,3] Therefore, estimation of PK-PD parameters of calcium channel blockers in individual hypertensive patients may allow us to gain further insight

into the relationship between antihypertensive efficacy of calcium channel blockers and plasma renin levels.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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