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Original article

Comparison of different pharmacodynamic models for PK–PD modeling of verapamil in renovascular hypertension

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ABSTRACT

Introduction: The aim of this work was to compare the suitability of different pharmacodynamic models for PK–PD modeling of verapamil cardiovascular effects in aortic coarctated rats (ACo), a model of renovascular hypertension. **Methods:** A "shunt" microdialysis probe was inserted in a carotid artery of anaesthetized sham-operated (SO) and ACo rats for determination of verapamil plasma concentrations and their effects on blood pressure and heart rate after intravenous application (1 and 3 mg kg^{-1}). Correlation between verapamil plasma levels and their cardiovascular effects was established by fitting data to a linear, and a conventional and modified E_{max} model. **Results:** No differences in verapamil volume of distribution were observed between experimental groups. Whilst clearance increased with dose in SO rats, no differences were found in verapamil clearance in ACo comparing both dose levels. A good correlation between verapamil plasma unbound concentrations and their hypotensive and chronotropic effects was found in both experimental groups using the tested PK–PD models. Although all pharmacodynamic models allowed a precise estimation of verapamil PK–PD parameters, linear and E_{max} model did not permit an accurate PK–PD parameter estimation for the hypotensive and chronotropic effect, respectively. Conversely, the modified E_{max} model allows both a precise and accurate estimation of PK–PD parameters for verapamil effects. Although, absolute verapamil blood pressure lowering effect was greater in ACo rats compared with SO rats, no differences were found in verapamil PK–PD parameters estimated for the hypotensive response. **Discussion:** Side-by-side comparison of the tested pharmacodynamic models showed that accuracy of PK–PD parameters estimation by using the linear and classical E_{max} model depends on the magnitude of concentration–effect curve covered in the study. Conversely, the modified E_{max} model allowed both a precise and accurate estimation of PK–PD parameters, suggesting that the modified E_{max} pharmacodynamic model is the most suitable for verapamil PK–PD modeling.

1. Introduction

Drug therapy of hypertension might be improved by means of increasing current knowledge of pharmacokinetic–pharmacodynamic (PK–PD) properties of antihypertensive drugs (Donnelly, Elliott, & Meredith, 1992; Harder, Thürmann, & Rietbrock, 1994). PK–PD modeling of antihypertensive drugs in both basic and clinical research could contribute to drug development and clinical practice, considering that it allows a profound evaluation of the efficacy and safety of investigational antihypertensive agents, enhancement of preclinical information during the development process, and identification of factors that contribute to drug response variability. It also allows a rapid identification of poor or non-responders to drug therapy, and is useful to determine optimal antihypertensive drug and dose requirements in each hypertensive patient (Donnelly et al., 1992; Harder et al., 1994).

Calcium channel blockers, such as verapamil, interfere with L-calcium channels in the vascular smooth muscle and at the myocardium (Hoffman, 2006). It has been reported that there was a relationship between plasma concentrations of calcium channel blockers and their cardiovascular effects (Donnelly et al., 1992; Elliot & Jamali, 1999; Harder et al., 1994; Meredith, 1997), consistent with their reversible mode of action.

PK–PD modeling of the antihypertensive effect of verapamil has been extensively studied in both laboratory animals and in normotensive and hypertensive patients (Aarons, Baxter, & Gupta, 2004; Gupta et al., 2002; Harder et al., 1992; Harder, Reitbrock, & Thürman, 1993). Different pharmacodynamic models, including the linear and the E_{max} model, have been used to describe the relationship between

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verapamil plasma concentrations and their blood pressure lowering effect (Aarons et al., 2004; Gupta et al., 2002; Harder et al., 1992, 1993). Nevertheless, considering that it is necessary to reach the maximal effect after drug administration in order to apply a conventionally pharmacodynamic model (Toutain, 2002); further studies are needed to adequately estimate verapamil PK–PD parameters. In clinical and basic pharmacology, it is usual that the maximum effect of a compound will be unknown owing to safety concerns. To apply in this kind of experimental conditions, an alternative PK–PD model for data analysis was designed by Schoemaker, van Gerven, and Cohen (1998). The authors replaced the concentration yielding half-maximal response (EC_{50}) with S_0 (initial sensitivity to the drug) in the E_{max} equation, allowing a better estimation of PK–PD parameters.

Another controversy regarding calcium channel blockers is that these antihypertensive agents might not be effective in the treatment of high-renin hypertension (Resnick, 1986, 1989). Although the antihypertensive efficacy of calcium channel blockers inversely correlates with plasma renin levels (Resnick, 1986), these drugs have been found to be effective in renovascular hypertension, a secondary form of hypertension characterized by high plasma renin activity (Rosenthal, 1993).

In accordance, the aim of the present work was to compare the suitability of different pharmacodynamic models for PK–PD modeling of the cardiovascular effects of verapamil in aortic coarctated rats, a well known animal model of renovascular hypertension with high-renin activity (Badyal, Lata, & Dadhich, 2003; Santos, Pontieri, Leomil Neto, & Michelini, 1995).

2. Methods

2.1. Induction of hypertension

Male Wistar rats were used (250–300 g). Animal experiments were performed in accordance with the "Principles of laboratory animal care" (NIH publication No. 85-3, revised 1985). Abdominal aortic coarctation (ACo) was carried out according to Rojo-Ortega and Genest (1968) in rats anaesthetized with ether. The technique consists of banding the aorta between the two renal arteries. Control animals were sham-operated (SO). Experiments were carried out 5 days after 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 verapamil at a dose of 1 and 3 mg kg^{-1}. A validated "shunt" microdialysis probe with one vascular inlet and two vascular outlets (Höcht, Opezzo, & Taira, 2003) was used for examining the time course of free verapamil 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 Spectramed P23XL pressure transducer (Spectramed, Oxnard, CA, USA) coupled to a Grass 79D polygraph (Grass Instrument, Quincy, MA, USA). 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, USA). The flow rate was 2 µl min^{-1} and samples were collected at 15-minute intervals.

After placement of the microdialysis probe, the *in vivo* recovery of verapamil was evaluated in all experiments using reverse microdialysis (Höcht, Opezzo, & Taira, 2007) by perfusing the microdialysis probe with a solution of verapamil (2 µg ml^{-1}) and by taking the proportion of lost across the dialysis membrane as an estimate of the recovery.

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

$$R = \frac{(C_{in} - C_{out})}{C_{in}}$$

where R is the verapamil *in vivo* recovery, C_{in} is the concentration of verapamil in the perfusate and C_{out} is the concentration of verapamil in the dialysate. Recovery of verapamil in all experiments was 0.31 ± 0.04.

After determination of the *in vivo* recovery, basal values of MAP and HR were determined during a 30-minute interval, followed by intravenous administration of 1 and 3 mg kg^{-1} of verapamil. For drug administration, verapamil (Droguerías Saporiti, Buenos Aires, Argentina) was dissolved in Ringer solution and administered during 2 min. MAP and HR were monitored along 2 h after drug administration with the simultaneous recollection of microdialysate samples every 5 min during the first 20-minute interval and every 15 min afterwards. Animals 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.

2.3. Analytical determination of verapamil in dialysate samples

Verapamil dialysate levels were determined by high-performance liquid chromatography (HPLC) with fluorometric 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, USA) and a fluorometric detector (FL-45A, Linear Instruments, Reno, USA).

The excitation and emission wavelength were 232 nm and 308 nm, respectively. Composition of the mobile phase was distilled water, acetonitrile, triethanolamine (60:40:0.2), 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 quantification of verapamil was 5.0 ng ml^{-1}. Linearity of the analytical method was determinate in the range of 5–1000 ng ml^{-1}.

2.4. Analysis of data

2.4.1. Correction of microdialysis data

To determine blood-unbound verapamil concentrations from the microdialysis data, drug concentrations in the microdialysis samples were adjusted using the *in vivo* probe recovery. So, the unbound verapamil concentrations in blood (C) were calculated using the following equation:

$$C_u = \frac{C_{out}}{R}$$

where C_u is the calculated unbound verapamil concentration, C_{out} is verapamil 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 (Ståhle, 1992). Therefore, the microdialysis data must be transformed from a series of integrals to a series of points corresponding at the end time of the sample interval, as previously reported (Bertera et al., 2007).

2.4.2. Verapamil pharmacokinetics

Compartment analysis of verapamil pharmacokinetics was used. The temporal profile of verapamil 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 verapamil levels vs. time (from 0 to infinity) was calculated using the trapezoidal rule. Clearance (Cl) and steady state volume of distribution (V_{dss}) were calculated by standard methods (Gibaldi & Perrier, 1982).

2.5. Pharmacokinetic–pharmacodynamic modeling of verapamil cardiovascular effect

In the verapamil pharmacokinetic–pharmacodynamic relationship study, the serum dialysate verapamil concentration and changes in MAP and HR were used. Considering that no time delay between the plasma concentrations of verapamil and their cardiovascular response was observed (Fig. 1), unbound plasma concentrations were directed linked to the pharmacological response (Csajka & Verotta, 2006). The PK–PD model used to simulate cardiovascular effects of verapamil was based on a two-compartment pharmacokinetic model with the pharmacological response directly linked from the central compartment.

In the present work, a side-by-side comparison of the different pharmacodynamic models reported for PK–PD modeling of verapamil, such as the linear and E_{max} model, with the modified E_{max} model was made.

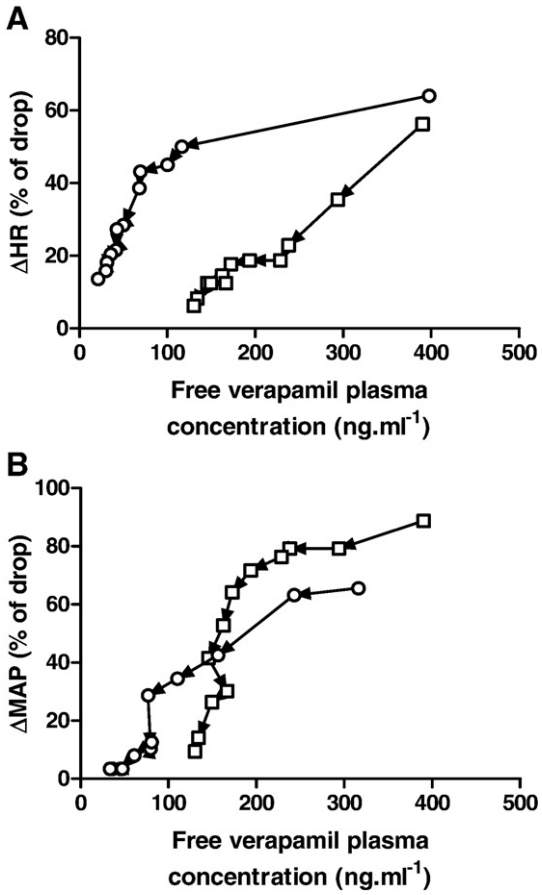

Fig. 1. Plotting of the chronotropic (in A) and blood pressure lowering effect (in B) of verapamil as a function of verapamil plasma concentrations in a representative animal of sham-operated (SO) rats (circles) and aortic coarctated (ACo) rats (squares). Data are connected in a chronological order.

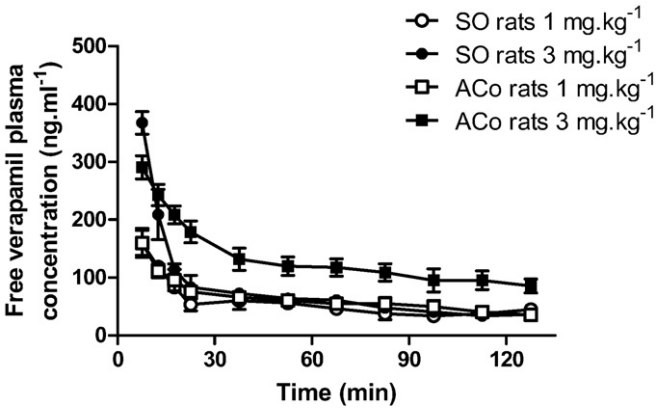

Fig. 2. Mean concentration values of verapamil vs. time in corrected plasma dialysate of sham-operated (SO) rats (circles) and aortic coarctated (ACo) rats (squares) after i.v. administration of verapamil (1 mg kg^{-1}, white symbols; 3 mg kg^{-1}, black symbols). Each point shows the mean ± SEM of six rats.

PK–PD modeling of verapamil chronotropic and blood lowering effect was carried out using the ADAPT II software package (D'Argenio & Schumitzky, 1997) by applying the maximum likelihood method as estimation procedure of PK–PD parameters. Data were adjusted to the following equations:

$$\text{Linear model}: E = \text{slope}^{*}C(t)$$

$$E_{\max}\ \text{model}: E = \left[\frac{E_{\max}{}^{*}C(t)}{EC_{50} + C(t)}\right]$$

$$\text{Modified } E_{\max}\ \text{model}: E = \left[\frac{S_0{}^{*}E_{\max}{}^{*}C(t)}{E_{\max} + S_0{}^{*}C(t)}\right]$$

where E is the drop in HR and MAP, EC_{50} is the verapamil concentration yielding half-maximal response, S_0 is the initial sensitivity to the drug, E_{max} is the maximal response, and $C(t)$ is the verapamil plasma concentration at time t. As relative response was used for PK–PD simulations, E_{max} was constrained to be less or equal than 100%.

2.6. 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 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. 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 ANOVA and the test of Bonferroni as the post-hoc test.

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

3. Results

Basal values of MAP and HR were 90.6 ± 3.5 mm Hg and 425 ± 8 bpm ($n=12$), respectively, in anaesthetized SO animals and 108.9 ± 4.5 mm Hg ($p<0.05$ vs. SO rats) and 453 ± 6 bpm ($p<0.05$ vs. SO rats) ($n=12$) respectively in anaesthetized ACo rats.

3.1. Pharmacokinetics of verapamil

Fig. 2 shows the verapamil concentration–time profile obtained from the microdialysis corrected data from SO rats ($n=6$) and ACo

Table 1
Pharmacokinetic parameters of verapamil obtained from dialysate samples: AUC (area under the curve), β (constant of elimination), α (constant of distribution), Cl (clearance), and V_{dss} (volume of distribution at the steady state) in sham-operated (SO) and aortic coarctated (ACo) rats after the i.v. administration of drug (1 mg kg^{-1} and 3 mg kg^{-1}).

Pharmacokinetic parameter	SO rats		ACo rats	
	1 mg kg^{-1}	3 mg kg^{-1}	1 mg kg^{-1}	3 mg kg^{-1}
α (h^{-1})	9.8±1.8	12.5±1.7	9.4±1.9	9.7±2.5
β (h^{-1})	0.48±0.10	0.63±0.09	0.33±0.05	0.27±0.05*
Cl (ml min^{-1})	119±30	215±31#	79 ±14	92±13*
V_{dss} (l)	12.8±1.7	16.1±1.5	14.5±1.7	17.5±2.1
AUC (ng ml^{-1} h^{-1})	203±52	287±47	202±25	595±80*

Data are expressed as mean±SEM of six animals.
* $p<0.05$ vs. SO rats.
\# $p<0.05$ vs. 1 mg kg^{-1} verapamil.

animals ($n=6$) after i.v. administration of 1 mg kg^{-1} and 3 mg kg^{-1} of the drug. A biexponential decay of plasma verapamil levels was found in all experiments compatible with a two-compartment pharmacokinetic model. Moreover, data fitted better to a two-compartment model (AIC=68.7) with regard to a mono-compartment pharmacokinetic model (AIC=88.1). At the higher dose, plasma concentrations of verapamil were significantly higher in ACo rats with regards to SO animals (Fig. 2).

The resulting pharmacokinetic parameters are shown in Table 1. Maximal serum concentration (C_{max}) of verapamil was similar between both experimental groups, and therefore no changes in the volume of distribution (V_{dss}) were found in ACo animals compared with normotensive SO rats (Table 1). Pharmacokinetics of verapamil shows a non-linear profile in SO animals, considering that verapamil clearance was significantly greater after administration of 3 mg kg^{-1} of the drug with regard to the lower dose. Conversely, clearance of verapamil was dose independent in hypertensive ACo rats (Table 1). At the higher dose, plasma clearance of verapamil was significantly lower in ACo rats compared with the normotensive group.

Finally, a proportional increase of AUC with dose increment was observed in ACo animals but not in SO rats.

3.2. PK–PD modeling of the chronotropic effect of verapamil

Fig. 3 shows the time course of HR after i.v. administration of verapamil 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 bradychardic effect of verapamil was observed in SO rats (1 mg kg^{-1}: ΔHR: −27±5%, $n=6$; 3 mg kg^{-1}: ΔHR: −42±3%, $n=6$) and ACo animals (1 mg kg^{-1}: ΔHR: −31±3%, $n=6$; 3 mg kg^{-1}: ΔHR: −53±4%, $n=6$) (Fig. 3). Chronotropic effect of verapamil was similar comparing both experimental groups. The application of a higher dose of verapamil (10 mg kg^{-1}) induced sinus arrest in both experimental groups (data not shown).

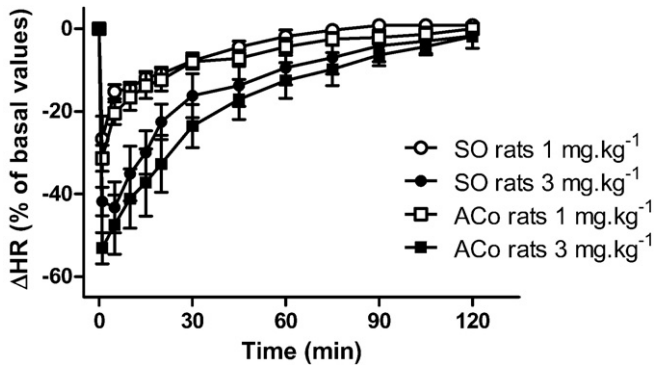

Fig. 3. Time course of the change of heart rate (ΔHR, % of basal heart rate), after i.v. administration of verapamil (1 mg kg^{-1}, white symbols; 3 mg kg^{-1}, black symbols) in sham-operated (SO) rats (circles) and aortic coarctated (ACo) rats (squares). Each point shows the mean±SEM of six animals.

Table 2
Comparison of goodness of fit of pharmacokinetic–pharmacodynamic simulations of verapamil chronotropic effect, by means of the Akaike Information Criterion (AIC), of the linear, E_{max} and modified E_{max} model in sham-operated (SO) rats and aortic coarctated (ACo) animals

Pharmacodynamic model	SO rats		ACo rats	
	1 mg kg^{-1}	3 mg kg^{-1}	1 mg kg^{-1}	3 mg kg^{-1}
Linear	38.52	46.17	37.31	47.26
E_{max}	38.75	42.56	34.34	48.82
Modified E_{max}	39.14	42.36	34.23	48.82

Data are expressed as mean of six animals.

As shown in Table 2, no differences were found in the goodness of fit of PK–PD simulations of verapamil chronotropic effect using the linear, E_{max} and modified E_{max} model. Table 3 shows PK–PD parameters estimation for the chronotropic effect of verapamil using the linear, E_{max} and modified E_{max} pharmacodynamic model. No differences were observed in the maximal chronotropic effect comparing both experimental groups. Sensitivity to verapamil chronotropic effect, expressed as slope, EC_{50} or S_0, was similar comparing SO and ACo animals (Table 3). On the other hand, slope and S_0 estimation did not change with verapamil dose in both experimental groups. Conversely, a dose dependent decrease of EC_{50} was observed in SO animals but not in ACo rats (Table 3), suggesting that the E_{max} model is not adequate for PK–PD modeling of the chronotropic effect of verapamil.

Estimation of PK–PD parameters by application of the linear, E_{max} and modified E_{max} model showed similar intersubject variability, considering that the estimation of slope, EC_{50} and S_0 showed comparable coefficient of variation (Table 3).

3.3. PK–PD modeling of the hypotensive effect of verapamil

The time course of MAP after i.v. administration of verapamil (1 mg kg^{-1} or 3 mg kg^{-1}) in SO animals and ACo rats is depicted in Fig. 4. Change of MAP was expressed as absolute change in MAP (in Fig. 4A) and as percentage of basal value during the 30 min before administration of the drug (in Fig. 4B). A dose dependent increase of the blood lowering effect of verapamil was observed in both experimental groups (Fig. 4). Although verapamil induced a greater hypotensive effect, expressed as absolute change, in ACo rats (1 mg kg^{-1}: ΔMAP: −58±3 mm Hg, $n=6$, $p<0.05$ vs. SO rats; 3 mg kg^{-1}: ΔMAP: −78±2 mm Hg, $n=6$, $p<0.05$ vs. SO rats) compared with SO animals (1 mg kg^{-1}:

Table 3
Side-by-side comparison of pharmacokinetic–pharmacodynamic parameters estimation of verapamil chronotropic effect obtained from sham-operated (SO) and aortic coarctated (ACo) rats by the application of the linear, E_{max} and modified E_{max} model

Pharmacokinetic–pharmacodynamic parameter	SO rats		ACo rats	
	1 mg kg^{-1}	3 mg kg^{-1}	1 mg kg^{-1}	3 mg kg^{-1}
E_{max} (%)	86 (35)	81 (26)	85 (34)	95 (14)
Slope (% ml ng^{-1})	140 (33)	164 (45)	177 (49)	188 (61)
S_0 (% µg^{-1} ml)	181 (40)	245 (42)	265 (22)	273 (65)
EC_{50} (ng ml^{-1})	555 (34)	264 (39)*	297 (46)	423 (68)

E_{max}: Maximal response, S_0: initial sensitivity to the chronotropic effect of verapamil, EC_{50}: effective concentration yielding half-maximal response.
Data are expressed as mean±SEM of six animals.
* $p<0.05$ vs. 1 mg kg^{-1} verapamil.

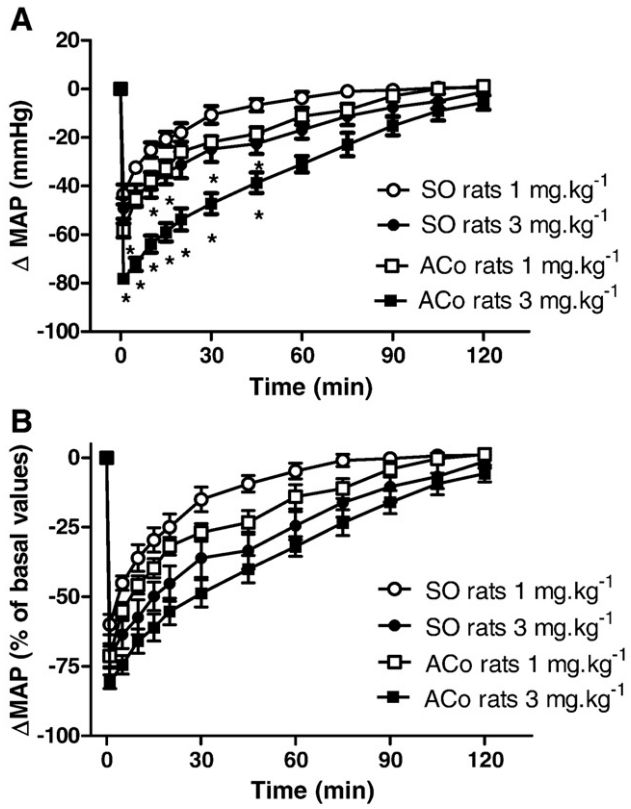

Fig. 4. Time course of the change of mean arterial pressure (ΔMAP), expressed as absolute change (A) and relative change to basal values (B), after i.v. administration of verapamil (1 mg kg^{-1}, white symbols; 3 mg kg^{-1}, black symbols) in sham-operated (SO) rats (circles) and aortic coarctated (ACo) rats (squares). Each point shows the mean ± SEM of six animals. *$p<0.05$ vs. SO rats.

ΔMAP: −43±4 mm Hg, $n=6$; 3 mg kg^{-1}: ΔMAP: −49±4 mm Hg, $n=6$); relative blood pressure effect of verapamil was not different between SO (1 mg kg^{-1}: ΔMAP: −60±4%, $n=6$; 3 mg kg^{-1}: ΔMAP: −71±4%, $n=6$) and ACo rats (1 mg kg^{-1}: ΔMAP: −71±4%, $n=6$; 3 mg kg^{-1}: ΔMAP: −80±3 %, $n=6$).

As shown in Table 4, no differences were found in the goodness of fit of PK–PD simulations of verapamil hypotensive effect using the linear, E_{max} and modified E_{max} model. The obtained PK–PD parameters for the hypotensive effect of verapamil after administration of 1 and 3 mg kg^{-1} in SO and ACo animals are shown in Table 5. No differences were found in the maximal response comparing SO and ACo rats (Table 5). Sensitivity to verapamil hypotensive effect, expressed as slope, EC_{50} or S_0, was similar comparing SO and ACo animals (Table 5). On the other hand, EC_{50} and S_0 estimation did not change with verapamil dose in both experimental groups. Conversely, a dose dependent decrease of slope was observed in SO and ACo animals

Table 4
Comparison of goodness of fit of pharmacokinetic–pharmacodynamic simulations of verapamil hypotensive effect, by means of the Akaike Information Criterion (AIC), of the linear, E_{max} and modified E_{max} model in sham-operated (SO) rats and aortic coarctated (ACo) animals

Pharmacodynamic model	SO rats		ACo rats	
	1 mg kg^{-1}	3 mg kg^{-1}	1 mg kg^{-1}	3 mg kg^{-1}
Linear	46.58	52.41	41.85	54.56
E_{max}	47.31	50.86	44.15	55.73
Modified E_{max}	46.45	51.72	44.36	56.15

Data are expressed as mean of six animals.

Table 5
Side-by-side comparison of pharmacokinetic–pharmacodynamic parameters estimation of verapamil hypotensive effect obtained from sham-operated (SO) and aortic coarctated (ACo) rats by the application of the linear, E_{max} and modified E_{max} model

Pharmacokinetic–pharmacodynamic parameter	SO rats		ACo rats	
	1 mg kg^{-1}	3 mg kg^{-1}	1 mg kg^{-1}	3 mg kg^{-1}
E_{max} (%)	96 (19)	98 (22)	95 (19)	98 (21)
slope (% ml ng^{-1})	382 (30)	245 (43)*	458 (37)	302 (26)*
S_0 (% μg^{-1}.ml)	784 (34)	773 (44)	859 (32)	837 (28)
EC_{50} (ng ml^{-1})	147 (37)	131 (36)	144 (30)	127 (32)

E_{max}: Maximal response, S_0: initial sensitivity to the chronotropic effect of verapamil, EC_{50}: effective concentration yielding half-maximal response.
Data are expressed as mean ± SEM of six animals.
* $p<0.05$ vs. 1 mg kg^{-1} verapamil.

(Table 5), suggesting that the linear model is not adequate for PK–PD modeling of the hypotensive effect of verapamil. Estimation of PK–PD parameters by application of the linear, E_{max} and modified E_{max} model showed similar intersubject variability, considering that the estimation of slope, EC_{50} and S_0 showed comparable coefficient of variation (Table 5).

4. Discussion

In the present work, side-by-side comparison of different pharmacodynamic models for PK–PD modeling of verapamil has shown that although all pharmacodynamic models allow a precise estimation of PK–PD parameters, the linear and E_{max} model did not permit an accurate PK–PD parameter estimation for the hypotensive and chronotropic effect, respectively. Conversely, the modified E_{max} model allows both a precise and accurate estimation of PK–PD parameters for verapamil cardiovascular effects, suggesting that the modified E_{max} model seems to be the most adequate pharmacodynamic model for PK–PD modeling of verapamil. In addition, no differences were found in the relative blood pressure lowering effect of verapamil in 5 days ACo animals with regard to SO rats, indicating that verapamil is at least equally effective in reducing blood pressure in high-renin hypertensive rats compared to normotensive animals.

Pharmacokinetic properties of verapamil were also evaluated in SO and ACo animals using the microdialysis technique. Microdialysis sampling has been extensively used for the evaluation of plasma concentrations of therapeutic agents in basic research (Höcht, Opezzo, & Taira, 2004), considering that this technique allows a continuous monitoring of unbound plasma concentrations of drugs without fluid removal. Previously, we have demonstrated the utility of our shunt microdialysis intraarterial probe for the study of pharmacokinetics of other antihypertensive drugs, including metoprolol (Höcht, Di Verniero, Opezzo, & Taira, 2005), irbesartan (Höcht et al., 2003) and diltiazem (Bertera et al., 2007).

Verapamil pharmacokinetic properties have been extensively studied in both clinical and basic research (Echizen & Eichelbaum, 1986; Eichelbaum, Somogyi, von Unruh, & Dengler, 1981; Hamann, Blouin, & McAllister, 1984; Mori et al., 2001; McAllister, Schloemer, & Hamann, 1986). However, to the best our knowledge, pharmacokinetic properties of verapamil were not evaluated in animal models of hypertension.

Verapamil undergoes extensive hepatic biotransformation mediated by CYP3A4 isoenzyme showing a hepatic extraction fraction of 0.86 in normotensive rats and humans (Manitpisitkul & Chiou, 1993; Woodcock, Schulz, Kober, & Rietbrock, 1981). In addition, verapamil is 95% bound to plasma proteins (Keefe, Yee, & Kates, 1981).

In the present work, we studied verapamil pharmacokinetics in urethane-chloralose anaesthetized SO and ACo rats. Meneguz, Fortuna, Lorenzini, and Volpe (1999) and Loch, Potter, and Bachmann

(1995) 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 verapamil pharmacokinetics in our experimental conditions, considering that systemic plasma clearance of verapamil 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. (1995) and Meneguz et al. (1999) in their previous reports.

Verapamil pharmacokinetic study describes a non-linear pharmacokinetic profile in SO rats but not in ACo animals. Moreover, clearance of verapamil was significantly lower in ACo animals with regard to SO rats after administration of 3 mg kg^{-1} of the drug. Taking into account that hepatic metabolic rate of verapamil depends on hepatic blood flow, an increase of verapamil dose could produce a greater hepatic perfusion due to vasodilatation, enhancing drug biotransformation. In accordance, although changes in hepatic blood flow by verapamil administration were not directly evaluated in the present study, Meredith, Elliot, Pasanisi, Kelman, Sumner and Reid (1985) have demonstrated an enhanced hepatic perfusion due to verapamil administration.

Conversely, hepatic clearance of verapamil showed a dose independent pattern in hypertensive ACo rats. Although further studies are needed, profound morphological changes were previously found in hepatic vessels of rats with aortic coarctation that could affect hepatic perfusion (Novikov, Shormanov, & Kulikov, 2006; Shormanov & Kulikov, 2003).

The main objective of the present work was to compare the suitability of different pharmacodynamic models for PK–PD modeling of cardiovascular effects of verapamil. Although several studies have described a good relationship between verapamil plasma concentrations and their cardiovascular effects (Aarons et al., 2004; Gupta, Modi, Sathyan, Ho Pl, & Aarons, 2002; Harder et al., 1992, 1993), to date there is no consensus regarding the most adequate PK–PD model. In this way, the linear and the E_{max} pharmacodynamic model were equally used to describe PK–PD relationship of verapamil (Aarons et al., 2004; Gupta et al., 2002; Harder et al., 1992, 1993). Although most PK–PD studies directly related verapamil plasma concentrations to their pharmacological response, Harder, Rietbrock and Thürmann (1993) also used a PK–PD model with an effect compartment to justify a possible delay in the onset of the cardiovascular effect of verapamil. However, present PK–PD studies of verapamil have some methodological limitations. For instance, it is a well known fact that an accurate estimation of PK–PD parameters using the E_{max} model needs the determination of the complete pharmacodynamic range after application of a single dose of the drug (Toutain, 2002). When the E_{max} model is used to estimate a curve without a clear maximum, E_{max} and EC_{50} estimates are extremely variable while their ratio is far less so (Schoemaker et al., 1998). Schoemaker et al. (1998) designed a modified pharmacodynamic model by replacing the parameter E_{max}/EC_{50} 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 second place, although most studies found a good PK–PD correlation after applying the linear model, this pharmacodynamic model has several pitfalls, especially by considering the absence of a maximal response (Pérez-Urizar, Granados-Soto, Flores-Murrieta, & Castañeda-Hernández, 2000). In addition, although in most clinical PK–PD studies different dose levels of verapamil were used, no comparisons of PK–PD parameters of verapamil estimated from the different applied doses were made (Aarons et al., 2004; Gupta et al., 2002; Harder et al., 1992, 1993). Therefore, it seems necessary to further validate PK–PD modeling of cardiovascular effects of verapamil.

In the present work, we compared PK–PD parameter estimation of verapamil by using traditional pharmacodynamic models, such as the linear model and E_{max} model, with the modified E_{max} model. Free verapamil plasma concentrations were directly related to the chronotropic and blood pressure lowering effect of the drug without the use of a link model. As shown in Fig. 1, our PK–PD analysis demonstrated the absence of time delay between plasma concentrations of verapamil and their cardiovascular effects. This finding is supported by the fact that verapamil blocks L-calcium channel in both smooth muscle of arterial vessels and in the myocardium. Therefore, verapamil acts on rapid responding receptors localized in high perfused tissues.

A good correlation between verapamil plasma concentrations and their cardiovascular effects was found for all tested pharmacodynamic models without differences between them. Similarity in the correlation parameters comparing the linear and the different E_{max} models could be explained by the fact that the concentration–effect curve of drugs remains relative linear between no effect and approximately 75% of the maximal response and only shows a clear hyperbolic relationship at response levels above 75%.

In addition, PK–PD parameter estimation showed similar intersubject variability comparing the 3 tested pharmacodynamic models, considering that the coefficient of variation was nearly similar after applying the different models.

However, differences were found in the accuracy of PK–PD parameter estimation using the linear, E_{max} and modified E_{max} model. Although the E_{max} and modified E_{max} model allowed an accurate estimation of verapamil sensitivity to the hypotensive effect, a dose dependent decrease in verapamil slope was found after applying the linear model in both experimental models. These results could be explained by the fact that, after administration of 3 mg kg^{-1} of verapamil, a higher degree of the concentration–effect curve is achieved including the initial part of the hyperbolic form of the concentration–effect curve. Therefore, as pharmacological response increases slower at the higher part of the concentration–response curve, a lower slope was obtained after administration of 3 mg kg^{-1} with regard to the lower dose. This concept is exemplified in a simulation shown in Fig. 5. According to our results, Harder et al. (1993) have found that the slope for the hypotensive effect of verapamil after administration of a single dose was significantly greater than the slope estimated after multiple dosing. Although in this report it was concluded that the existence of tolerance to verapamil blood pressure lowering effect was due to chronic exposure to the drug, the most plausible explanation is the fact that a higher degree of the concentration–response curve of verapamil may be achieved due to higher drug concentration at steady state. Therefore, this report clearly demonstrates that the use of inadequate PK–PD models could arise erroneous conclusions.

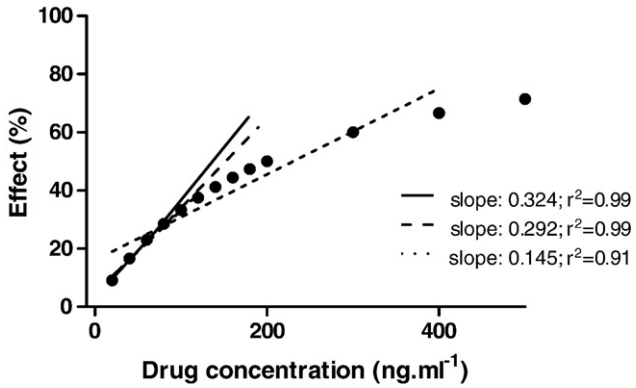

Fig. 5. Simulated concentration–effect curve (E_{max}: 100%, EC_{50}: 200 ng ml^{-1}) (circles) and linear analysis of the concentration–effect curve ranged between 0 and 30% of maximal effect (continuous line), linear analysis of the concentration–effect curve ranged between 0 and 45% of maximal effect (dotted line) and linear analysis of the concentration–effect curve ranged between 0 and 70% of maximal effect (fine dotted line).

Conversely, estimation of PK–PD parameters of verapamil showed dose independency after applying the E_{max} and modified E_{max} model, demonstrating the accuracy of PK–PD modeling of verapamil with these pharmacodynamic models. In a previous report (Bertera et al., 2007), we found that whilst estimation of diltiazem PK–PD parameters using the classical E_{max} model was inaccurate showing a dose dependency in EC_{50} estimation, the modified E_{max} model proposed by Shoemaker allows a precise and accurate estimation of diltiazem PK–PD parameters. Although the results of the present work are contradictory to the previous report, in our study a greater shape of the concentration–effect curve of verapamil hypotensive effect have been achieved after administration of both doses. Conversely, in the diltiazem study only half-maximal effect has been attained with the achieved diltiazem plasma concentrations (Bertera et al., 2007). Taking together, our results suggest that application of the classical E_{max} model only allows an accurate estimation of drug potency when more than 50% of the entire shape of a concentration–effect curve could be achieved after administration of a single dose.

Conversely, in the PK–PD study of verapamil chronotropic effect, while application of the linear and modified E_{max} model allowed an accurate estimation of verapamil sensitivity to the bradychardic effect, estimation of EC_{50} by using the classical E_{max} model showed a dose dependent pattern in SO rats. Estimation of the slope of verapamil chronotropic effect was not different comparing dose levels in both experimental groups, suggesting that the linear model, in our experimental conditions, seems to be suitable for PK–PD modeling of verapamil effect on heart rate. A possible explanation to this finding is the fact that only 50% of the entire shape of the concentration–effect curve to verapamil chronotropic effect has been achieved after administration of a single dose (Fig. 5).

When comparing the classical E_{max} and the modified E_{max} model for PK–PD modeling of verapamil chronotropic effect, only the E_{max} model designed by Schoemaker et al. (1998) allowed an accurate estimation of verapamil sensitivity. Conversely, potency, expressed as EC_{50}, decreased with dose increment in SO rats. Previously, a dose dependency in diltiazem potency estimation has also been found by applying the classical E_{max} model (Bertera et al., 2007). Therefore, the present results suggest again that the classical E_{max} model does not allow an accurate estimation of PK–PD parameters if only the lower part of the concentration–effect curve have been ranged after a single administration.

Finally, the present work also addressed the study of verapamil hypotensive efficacy in experimental renovascular hypertension. 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 (Resnick, 1986, 1989). However, controversy exists with regards to the efficacy of these 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 the clinical practice (Rosenthal, 1993), Zawada and Johnson (1984) showed the absence of blood pressure lowering effect of these drugs in experimental renovascular hypertension. Moreover, to the best our knowledge, although functionality of calcium channels have been extensively studied in ACo rats, no *in vivo* studies were made in order to establish blood pressure lowering efficacy of verapamil in ACo animals. *In vitro* studies have demonstrated that the increased vascular tone of aorta from ACo animals is a consequence of increased cytoplasmatic calcium levels (Storm & Webb, 1993). Moreover, a good correlation between calcium content and elevated blood pressure was found in rats with ACo (Yang & Nickerson, 1988). In the present work, according to previous reports (Donnelly, Reid, Meredith, Ahmed, & Elliott, 1988), verapamil hypotensive efficacy was related to pretreatment blood pressure, considering that ACo rats showed a greater absolute hypotensive effect to verapamil administration compared with SO animals. Conversely, relative blood pressure lowering effect of verapamil was similar comparing both experimental groups. In addition, PK–PD analysis showed that both sensitivity and efficacy of verapamil were not altered in ACo rats with regards to normotensive animals. Therefore, our results suggest that verapamil hypotensive effect is more related to pretreatment blood pressure levels than to renin plasma levels.

In conclusion, the results of the present work suggest alterations in verapamil clearance in ACo hypertensive animals. In addition, although absolute blood pressure lowering effect of verapamil was greater in ACo rats compared with normotensive SO rats, no differences were found in verapamil PK–PD parameters estimated for the hypotensive response. Finally, side-by-side comparison of the tested pharmacodynamic models showed that accuracy of PK–PD parameters estimation by using the linear and classical E_{max} model depends on the magnitude of the concentration–effect curve covered by the PK–PD study. Conversely, the modified E_{max} model allowed both a precise and accurate estimation of PK–PD parameters independently of the covered pharmacodynamic range, suggesting that the modified E_{max} pharmacodynamic model is the most suitable for PK–PD modeling of verapamil.

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