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Pharmacokinetic/pharmacodynamic modeling of benazepril and benazeprilat after administration of intravenous and oral doses of benazepril in healthy horses.

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Abstract

Pharmacokinetic and pharmacodynamic (PK/PD) properties of the angiotensin-converting enzyme inhibitor (ACEI) benazeprilat have not been evaluated in horses. This study was designed to establish PK profiles for benazepril and benazeprilat after intravenous (IV) and oral (PO) administration of benazepril using a PK/PD model. This study also aims to determine the effects of benazeprilat on serum angiotensin converting enzyme (ACE), selecting the most appropriate dose that suppresses ACE activity. Six healthy horses in a crossover design received IV benazepril at 0.50 mg/kg and PO at doses 0 (placebo), 0.25, 0.50 and 1.00 mg/kg. Blood pressures (BP) were measured and blood samples were obtained at different times in order to measure serum drug concentrations and serum ACE activity, using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and spectrophotometry, respectively. Systemic bioavailability of benazeprilat after PO benazepril was 3-4%. Maximum ACE inhibitions from baseline were 99.63% (IV benazepril), 6.77% (placebo) and 78.91%, 85.74% and 89.51% (for the three PO benazepril doses). Significant differences in BP were not found. Although oral availability was low, benazeprilat 1.00 mg/kg, reached sufficient serum concentrations to induce long lasting serum ACE inhibitions (between 88 and 50%) for the first 48 h. Additional research on benazepril administration in equine patients is indicated.

Keywords: Angiotensin-converting enzyme inhibitors; Benazeprilat; Benazepril; Pharmacokinetic-pharmacodynamic; Horses

1. Introduction

Benazepril is a pro-drug, which after *per os* (PO) absorption undergoes hydrolization to its active metabolite, benazeprilat. It is an angiotensin converting enzyme inhibitor (ACEI), as it prevents conversion of angiotensin I into angiotensin II (Toutain and Lefèbvre, 2004). Angiotensin II causes vasoconstriction, and therefore, benazeprilat and other ACEIs such as ramiprilat or enalaprilat are used to modulate blood pressure (BP) in human and veterinary medicine in pathological processes in which hypertension is a concern (Lefebvre et al., 2007). Considering this, the main clinical indications for ACEI administration are chronic heart failure, essential hypertension and chronic renal failure. Because of the clinical importance of these disorders in dogs and cats, there is a huge amount of information derived from clinical studies (reviewed by Lefebvre et al., 2007). However, studies of ACEIs in horses are scarce, with a progressively increasing number of reports being published in recent years (Afonso et al., 2013; Davis et al., 2014; Gómez-Díez et al., 2014; Muñoz et al., 2016; Serrano-Rodríguez et al., 2016). The interest in these pro-drugs has risen recently in horses because there is an increased awareness of cardiovascular abnormalities and their consequences in sport horses. In addition, new researchers have highlighted the clinical relevance of some hypertensive situations in equids. Five cases of hypertensive cardiomyopathy have been reported (Navas de Solís et al., 2013), and there are also indications that systemic hypertension might be a feature of equine metabolic disease, as ponies with this syndrome and previous laminitis have been reported to have elevated systemic BPs in summer (Bailey et al., 2008).

Pharmacokinetic and pharmacodynamic (PK/PD) studies of ACEIs in equine patients are limited to enalaprilat, quinaprilat and ramiprilat after intravenous (IV) and PO enalapril,

quinapril and ramipril, respectively (Gardner et al., 2004; Davis et al., 2014; Gómez-Díez et al., 2014; Serrano-Rodríguez et al., 2016). To the authors' knowledge, there are three papers regarding benazepril, another ACEI. In one paper, Afonso et al. (2013) administered two different PO doses of benazepril, considered as low (0.25 mg/kg) and high (0.50 mg/kg), extrapolated from the dosage recommended for dogs. These authors measured ACE inhibition and BP, but because they did not administer IV benazepril, only a pharmacodynamic analysis was made. Later, Muñoz et al. (2016) compared the effect of different ACEIs administered 2 h before exercise and the response of BP after an intense exercise. It was found that only benazepril was able to avoid the hypertensive response to exercise. More recently, Afonso et al. (2016) evaluated BP in response to angiotensin I administration after four different benazepril PO doses (0.5, 1, 2 and 4 mg/kg). They found that the attenuation of BP was modest despite achieving an adequate serum ACE inhibition. Although in these recent years the number of studies regarding benazepril has increased, pharmacokinetic analyses are still lacking. Moreover, the previously mentioned articles did not measure blood benazepril-benazeprilat concentrations. Additionally, the effects of multiple PO doses are also unknown.

Therefore, the current study aims to: (1) establish serum concentration–time profile for benazepril, and its active metabolite benazeprilat after IV benazepril at 0.50 mg/kg, PO (placebo), and three different PO benazepril doses (0.25, 0.50 and 1.00 mg/kg); (2) determine the effects of benazeprilat on serum ACE activity; (3) investigate the effects on BP with these doses and routes; and (4) predict and simulate the most appropriate PO dose that suppresses ACE activity after single and multiple PO doses of benazepril.

2.1. Material and methods

2.1. Horses

Six crossbred horses, two mares and four geldings, between 5 and 11 years (10.8±2.35), and weighing between 391 and 594 kg (477.44±67.58) were studied. Prior to the experiment, physical examinations, hematology, serum biochemistry, myocardial troponin concentrations, electrocardiography, echocardiography and BP measurements, were performed. Only healthy animals were studied, and they did not receive any other medication during the period of study.

The animals were located in individual paddocks and were fed with rye-grass haylage. The horses did not have access to salt or electrolyte supplements during the study.

2.2. Experimental protocol

The research was approved by the Ethical Committee for Animal Experimentation of the University of Córdoba (Reference: 55.60 PE, date of approval February 1st 2010).

The study was conducted in two trials. In the first trial, each animal received IV benazepril at 0.50 mg/kg. After a week of washout, the second trial was undertaken, using a blinded and randomized Latin square (6×4) design. Each animal received PO placebo or benazepril at 0.25, 0.50, or 1.00 mg/kg, with at least 1 week of washout between trials. Food was withdrawn from 12 h before administration of the pro-drug to 8 h after.

For IV study, benazepril (Sigma-Aldrich) was dissolved in 0.7% saline and 1% $NaHCO_3$ (King et al., 2003). For PO doses, benazepril hydrochloride tablets (Fortekor 20 mg,

Novartis) were dissolved and suspended in 150 mL of water, sonicated in an ultrasonic bath for 15 min and stored at 4 ºC before trials. After this dissolution process, benazepril concentration was tested by the analytical technique and benazeprilat was not detected. For placebo, the equivalent amount of water without the pro-drug was used. Pro-drugs and placebo were administered by nasogastric intubation at ambient temperature (between 20-24ºC approximately).

In each assay, venous blood samples were taken before administration (time 0), and at 5, 10, 15, 30 and 45 min and at 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48 and 72 h after.

2.3. Blood pressure monitoring

Systolic (SBP) and diastolic (DBP) BP measurements were made non-invasively with a multiparametric monitor (S/5 Datex-Ohmeda Compact) in the coccygeal artery. Three BP measurements were made at each sampling point and the results are presented as the means of the three measurements.

2.4. Blood processing and analysis

After extraction, blood samples were poured into tubes without anticoagulant, left to coagulate in a refrigerator for 30 min and centrifuged. Harvested serum was stored at -90ºC until analysis.

Serum ACE activity was measured by spectrophotometry (Afonso et al., 2013; Gómez-Díez et al., 2014; Serrano-Rodríguez et al., 2016). Linear calibration curves from 3 to 150 IU/l were obtained, and the correlations coefficients (r) were >0.99. Precision of the

technique expressed as mean, standard deviation and coefficient of variation were 25.10 ± 0.80 IU/l (3.18%) and 75.04 ± 4.29 IU/l (5.72%) for intra-assay validation, and 25.63 ± 1.04 (4.04%) and 75.89 ± 4.90 (6.45%) for inter-assay validation, respectively. The limits of quantification (LOQ) and detection (LOD) were 5 and 3 IU/l, respectively.

Serum concentrations of benazeprilat (CymitQuímica, Barcelona, Spain), benazepril and enalapril as internal standard (Sigma-AldrichQuímica, Madrid, Spain) were measured using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay (Yuan et al., 2008). A volume of 700 µl of horse serum was spiked with internal standard solution at 50 ng/ml, and then 1 ml of methanol was added. After shaking in an ultrasonic bath for 5 min, and centrifugation at 28300 g for 10 min at 4º C, supernatant was extracted, and 3 ml of ethyl acetate were added. The mixture was blended and centrifuged for 5 min at 2000 g. The upper organic layer was evaporated to dryness under N_2 at 40ºC for about 15 minutes. The residue was reconstituted in 100 µl of methanol and an aliquot of 5 µl was injected into the LC-MS/MS system. Linear calibrations of benazepril and benazeprilat between 0.1-500 ng/ml were obtained ($r>0.99$). Precision of the technique expressed as mean, standard deviation and coefficient of variation were 19.69 ± 1.04 ng/ml (5.27%) and 39.81 ± 1.44 ng/ml (3.62%) for benazepril and 4.04 ± 0.10 ng/ml (2.52%) and 8.13 ± 0.47 ng/ml (5.77%) for benazeprilat after intra-assay validation, respectively. For both analytes these values were 18.97 ± 1.06 ng/ml (5.57%) and 41.24 ± 2.56 ng/ml (6.22%), and 4.24 ± 0.32 ng/ml (7.55%) and 8.33 ± 0.47 ng/ml (5.63%) after inter-assay validation, respectively. The limits of detection (LOD) and quantitation (LOQ) for both drugs were 0.03 and 0.1 ng/ml, respectively (see chromatographic methodology in Supplement data).

2.5. Pharmacokinetic and pharmacodynamic analysis

ACEI drugs have nonconventional PKs due to nonlinear binding to ACE, the target enzyme, and because there are two ACE pools, circulating and non-circulating. Circulating ACE originates mainly from vascular endothelium, representing about 5-30% of ACE pool in different species (Toutain and Lefèbvre, 2004). Therefore, compartmental PK/PD models with binding parameters of ACEI to ACE are required to allow an appropriate interpretation of the different disposition phases of ACEI curves (Toutain et al., 2000).

For each animal, PK-PD data were analyzed using the models developed by Lees et al. (1989) with the approach of Picard-Hagen et al. (2001). The final selected model included one compartment for benazepril and one compartment for benazeprilat directly linked into a direct effect (Toutain and Lefebvre, 2004). Furthermore, for PO administration, a depot compartment was included (see figures and equations in PK/PD modeling in Supplement data).

The parameters estimated for PK modeling were: k_a, the first order absorption rate constant for benazepril; k_f, the elimination rate constant for benazepril by formation of benazeprilat; and V_c/F, the apparent volume of distribution with respect to bioavailability and bioconversion from benazepril. The systemic clearance for benazeprilat with respect to the bioavailability and bioconversion from benazepril was determined as $Cl/F = k_{10} \cdot V_c/F$, with k_{10} indicating the elimination rate constant of benazeprilat. The elimination half-life was obtained as $t_{½k10} = (\ln 2)/k_{10}$. The F value was calculated from the ratio of Cl of benazeprilat after IV benazepril and Cl/F of benazeprilat after PO benazepril (King et al., 2003). Ideally, IV benazeprilat would have been administered to determine V_c, Cl and F values. However,

the necessary amount for IV formulation was not available. Administration of IV benazepril, however, provides important information about the biotransformation to benazeprilat, as described for other ACEIs in horses (Davis et al., 2014; Serrano-Rodríguez et al., 2016).

Binding parameters of benazeprilat to ACE were: K_d, equilibrium dissociation constant that expresses the affinity for ACE; B_{max}, size of ACE pool and f_{circ}, fraction between circulating and non-circulating ACE. Circulating ACE was obtained as $P_{max}=B_{max}\cdot f_{circ}$. (Toutain and Lefebvre, 2004). However, it is necessary to note that benazeprilat is bound to ACE, or free, or non-specifically bound to albumin. Free and albumin-bound fractions are indistinguishable in these models, and free benazeprilat concentration used for PD modeling corresponded to free benazeprilat plus benazeprilat bound to albumin (Toutain et al., 2000).

For PD data, the observed effect was defined as the serum ACE activity at each time expressed as a percentage of the control before administration of the pro-drug. The estimated parameters were IC_{50}, free benazeprilat concentration required to produce 50% ACE inhibition, and γ coefficient, also known as Hill coefficient, which describes the steepness of the sigmoid curve. Other parameters obtained were maximum serum ACE inhibition between 0-12 h ($\%I_{max}$), time to reach $\%I_{max}$ ($T_{\%Imax}$), and serum ACE inhibition at 12, 24, 48 and 72 h ($\%I_{12h}$, $\%I_{24h,}$ $\%I_{48h}$ and $\%I_{72h}$, respectively).

Because a long-term therapy is indicated for ACEIs, comparative simulations of PO doses of benazepril from 0.25 to 8.00 mg/kg were made after single and repeated doses administered daily for seven days. Predictions were performed with parameters obtained with

PO benazepril at 1.00 mg/kg after PK-PD analysis. The software used for modeling was ADAPT 5 (D´Argenio et al., 2009), and the simulations were developed using Berkeley Madonna software (Krause and Lowe, 2014).

2.6. Statistical analysis

Because the data did not follow a Gaussian distribution, non-parametric tests were used. Data are presented as median with interquartile range. A Wilcoxon rank sum test was used to assess significant differences between IV and PO routes at 0.50 mg/kg. A Kruskal-Wallis test was used to evaluate significant differences between PO doses including placebo. When differences were found, a Wilcoxon rank sum test was used. The significance level was fixed at $p<0.05$ (Statgraphics Centurion XVI.I, StatPoint Technologies).

3. Results

No local or systemic adverse reactions were detected during or after IV and PO administrations. SBP and DBP remained statistically unchanged during the whole experiment.

Mean (±SD) serum concentrations of benazepril and benazeprilat after IV and PO benazepril and mean (±SD) serum ACE activities, expressed as a percentage of controls (values at time 0) from 0 to 72 h are outlined in Fig 1 and 2, respectively. PK-PD parameters obtained and mean values of $\%I_{max}$, $T_{\%Imax}$, and $\%I_{12h}$, $\%I_{24h}$, $\%I_{48h}$ and $\%I_{72h}$ for each administration route and dose are presented in Table 1 and 2, respectively. Neither IV nor PO benazepril administration resulted in significant changes in SBP and DBP, even at the times when maximum serum ACE inhibitions were attained.

Serum benazepril and benazeprilat concentrations were low and declined rapidly after IV administration of benazepril. Benazepril concentrations were lower than benazeprilat concentrations from 0.16 h (Fig. 1a), suggesting a rapid elimination by biotransformation to benazeprilat with a mean k_f value of 7.471/h (Table 1). The decreasing phase of benazeprilat is controlled by k_{10} and reflects the elimination process with a half-life of 0.60 h (36 min approximately). It is likely that during this phase, serum ACE would be saturated, explaining the high inhibition values, close to 99.63% between 0.08 and 3.00 h (Fig 2a). Moreover, the terminal phase observed for benazeprilat reflects the binding to ACE and is influenced by binding parameters and by k_{10} (Lefèbvre et al., 2006).

Following PO benazepril administration, the bioavailability of benazeprilat was low (3.28-4.30%). Maximum inhibition values of 78.91, 85.74 and 89.51% after PO benazepril at 0.25, 0.50 and 1.00 mg/kg were obtained, respectively, and these data were lower than IV benazepril values at 0.50 mg/kg (Fig 2). However, the inhibition after PO doses lasted longer than IV administration (Fig. 2), particularly with 0.50 and 1.00 mg/kg of benazepril from 12 to 72 h, without significant differences between them (Table 2).

Values for k_a of benazepril were low. The elimination of benazeprilat described by k_{10} and $t_{1/2k10}$ was not different between PO doses (p >0.05), unlike the IV values. Binding parameters derived from the model indicated that the ACE pool binding capacity B_{max} was between 80-160 nmol/l, and approximately 10-18% of the ACE pool was circulating (Table 1).The K_d value was in the range of 1.22 to 4.22 nmol/l.

For benazeprilat, IC_{50} values of 0.55 nmol/l were found at 24 h after IV benazepril at 0.5 mg/kg. For the doses of PO benazepril at 0.5 and 1.0 mg/kg, IC_{50} values ranged between 0.80 and 1.12 nmol/l and were obtained at times comprised between 48 and 60 h (Table 1). These data were consistent with the inhibition values observed in those times (Fig. 2). Moreover, γ values close to 0.65 were found.

For the simulations, the predicted effects on serum ACE activity after single and multiple PO doses of benazepril from 0.25 to 8.00 mg/kg once a day, for a week were calculated and shown in Fig.3.

4. Discussion

ACEI pro-drugs have been studied more intensely in horses in recent years, because of the greater awareness in relation to the relevance of some potentially pathologic hypertensive situations in equine medicine, including cardiovascular diseases and equine metabolic syndrome. Furthermore, heart valve regurgitation is associated with an activation of the renin angiotensin aldosterone axis (Gehlen et al., 2008). In a preliminary report, Costa et al. (2012) also found greater serum ACE activity in horses with exercise-induced pulmonary hemorrhage; therefore, it might be a promising marker of this condition. However, this together with the vasodilatory effects might lead to fraudulent administration of these pro-drugs despite the fact that they are a prohibited (benazepril) or controlled (enalapril) medication according to the FEI (Fédération Equestre Internationalle). Nevertheless, we have to consider that these types of drugs could be the cornerstone to treat and even to delay clinical progression in cardiopathic patients or in geriatric horses at risk of heart disease and failure.

The current study aims to define PK/PD properties of benazepril and compare these with other ACEIs previously studied in the horse. After PK/PD analysis following a physiologically based interpretation of the serum curves, PO absorption of benazepril was found to be low. This finding might indicate that most of the pro-drug administered is not absorbed and higher PO doses should be used compared to the IV dose in order to achieve the same ACE inhibition. Consequently, the oral bioavailability of benazepril in horses was low, near 4%, and similar to the values described by previous researchers with other ACEIs. In fact, values close to 2 – 6% for quinaprilat and ramiprilat, and even lower for enalapril have been published suggesting that oral absorption of ACEIs in horses is low (Davis et al., 2014; Gómez-Díez et al., 2014; Serrano-Rodríguez et al., 2016). Several hypotheses related to the enteral absorption of these kinds of drugs, and the influence of intestinal uptake by peptide transporter 1 (PepT1) compared to passive diffusion have been proposed to explain these findings (Davis et al., 2014; Gómez-Díez et al., 2014; Maxwell, 2015).

Despite the low oral bioavailability, benazepril had sufficient enteral absorption and bioconversion to benazeprilat to induce maximum serum ACE inhibitions close to 78-89% at doses from 0.25 to 1.00 mg/kg of benazepril at times between 2 to 4 h post-administration. Similar values have been reported with ramiprilat after PO ramipril at 0.4 and 0.8 mg/kg (76.13 – 84.27%) but lower with quinaprilat after PO quinapril at 0.25 and 0.5 mg/kg (47.11 – 53.43%) and enalaprilat after PO enalapril at 1.00 and 2.00 mg/kg (26.11 – 30.19%). These findings suggest that benazepril and ramipril, at the doses tested, produce a higher ACE inhibition than quinapril and especially enalapril (Davis et al., 2014; Gómez-Díez et al., 2014; Serrano-Rodríguez et al., 2016).

In relation to ACE-binding and PD results, the low K_d data obtained suggest that benazeprilat has a greater affinity for ACE and indicates a relatively shallow and long-lasting concentration effect relationship due to the low γ and IC_{50} values observed (Fig. 2). For that reason, inhibition values up to 50% between 48 and 72 h can be obtained for very low benazeprilat concentrations at 0.50 and 1.00 mg/kg of benazepril unlike other ACEIs such as enalaprilat, ramiprilat or quinaprilat (Gómez-Díez et al., 2014, Davis et al., 2014; Serrano-Rodríguez et al., 2016). At this point, it is necessary to indicate that Afonso et al. (2013) found that PO benazepril resulted in a greater serum ACE inhibition compared with quinapril, ramipril and peridonpril in horses, and no feeding effect was observed in any PD parameters, suggesting that benazepril could be more useful in equine patients than other inhibitors.

After simulation with single and multiple doses, a close examination of the curves showed that although serum ACE inhibition rose at higher doses, the effect was similar from 1.00 to 8.00 mg/kg and PO doses from 1.00 to 8.00 mg/kg were not statistically different between them (Fig.3a, 3b). The data derived from these simulations are consistent with the experimental results reported in the recent paper of Afonso et al. (2016). This group described the effect on BP in response to exogenous angiotensin I administration after single PO benazepril administrations from 0.50 to 4.00 mg/kg. It was observed that the greater ACE inhibition found with increasing doses of PO benazepril did not lead to a greater BP attenuation after angiotensin I administration. All of these results taken into consideration together might indicate that PO benazepril at 1.00 mg/kg would be useful in horses with hypertension. In another study, horses received four different PO ACEIs or placebo 2 h before being exposed to an intense exercise that resulted in systemic hypertension. It was

found that the increase in BP immediately after exercise reached values ~~over~~ above baseline of 67.6% (placebo experiment), 52.7% (enalapril 2 mg/kg), 43.1% (quinapril 1 mg/kg), 26.6% (ramipril 0.2 mg/kg) and 4.2% (benazepril 0.5 mg/kg) (Muñoz et al., 2016). These findings appear to indicate that benazepril might be more effective than other ACEIs in the management of hypertension in horses. In most of the studies, as with the present research, normotensive individuals do not experience evident changes in BP with the administration of these types of drugs (Davis et al., 2014; Gómez-Díez et al., 2014; Muñoz et al., 2016; Serrano-Rodríguez et al., 2016). This is an important concern because in humans, hypotension could appear with the use of these drugs, until response to treatment is evaluated and an individual dosage is established for the patient. This hypotension can even be accentuated with a simultaneous inclusion of diuretics in the therapy (Yusuf et al., 2000). It should be kept in mind that these drugs might also be administered in cardiopathic normotensive horses.

Despite the complexity of the PK/PD model used in this research, the results and simulations of the present research suggest that benazeprilat, after PO benazepril administration, could be the most promising ACEI in the horse in comparison to other drugs from the same class. However it is necessary to note that there are too few studies on the effects of ACEIs in equine patients unlike in small animals or humans. At this point, benazepril seems to be the most useful ACEI, but another such as ramipril, which has closer PK/PD relationships in horses with benazepril, could be another interesting option. Nevertheless, those are preliminary studies with healthy horses and further work in equine patients should be conducted to support the clinical utility of benazepril in horses.

Conclusions

The use of physiological compartmental PK/PD models allowed the pharmacological study of benazeprilat after IV and PO doses of benazepril in horses. This study shows that PO availability of benazepril in horses was low. Serum ACE activity was never suppressed by more than 89.51% after PO benazepril, unlike the IV data of 99.63%, using half the dose of PO administration. It would be interesting to test whether a serum ACE inhibition near 90%, as found in our study with PO benazepril at 1.00 mg/kg, would be sufficient to counteract vasoconstriction in pathological conditions. However, data presented in this research together with those reported by other groups indicate that clinical efficacy of benazepril might have promising results in equine patients.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Appendix A: Supplementary data

Supplementary data to this article can be found online at

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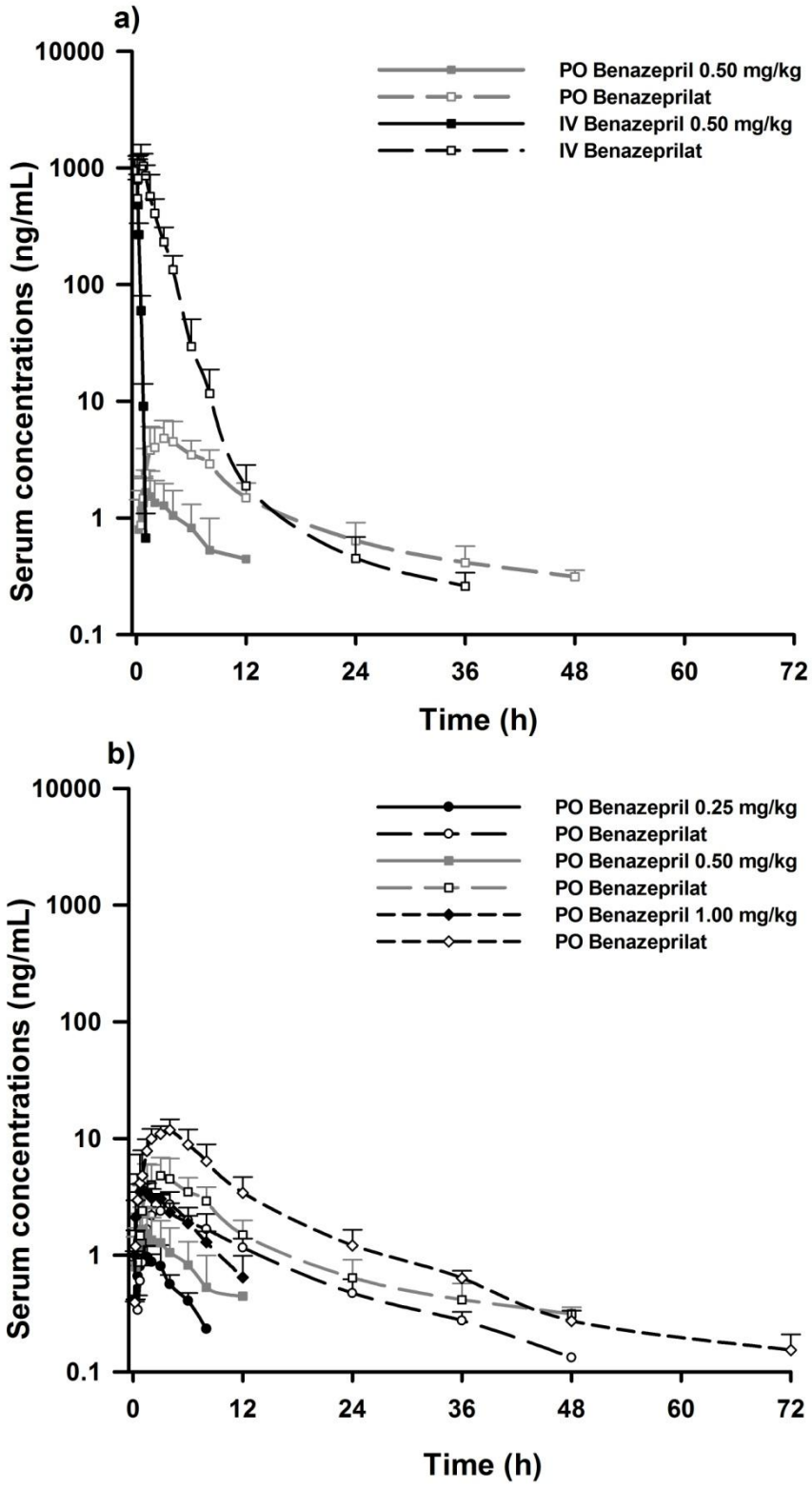

Fig. 1

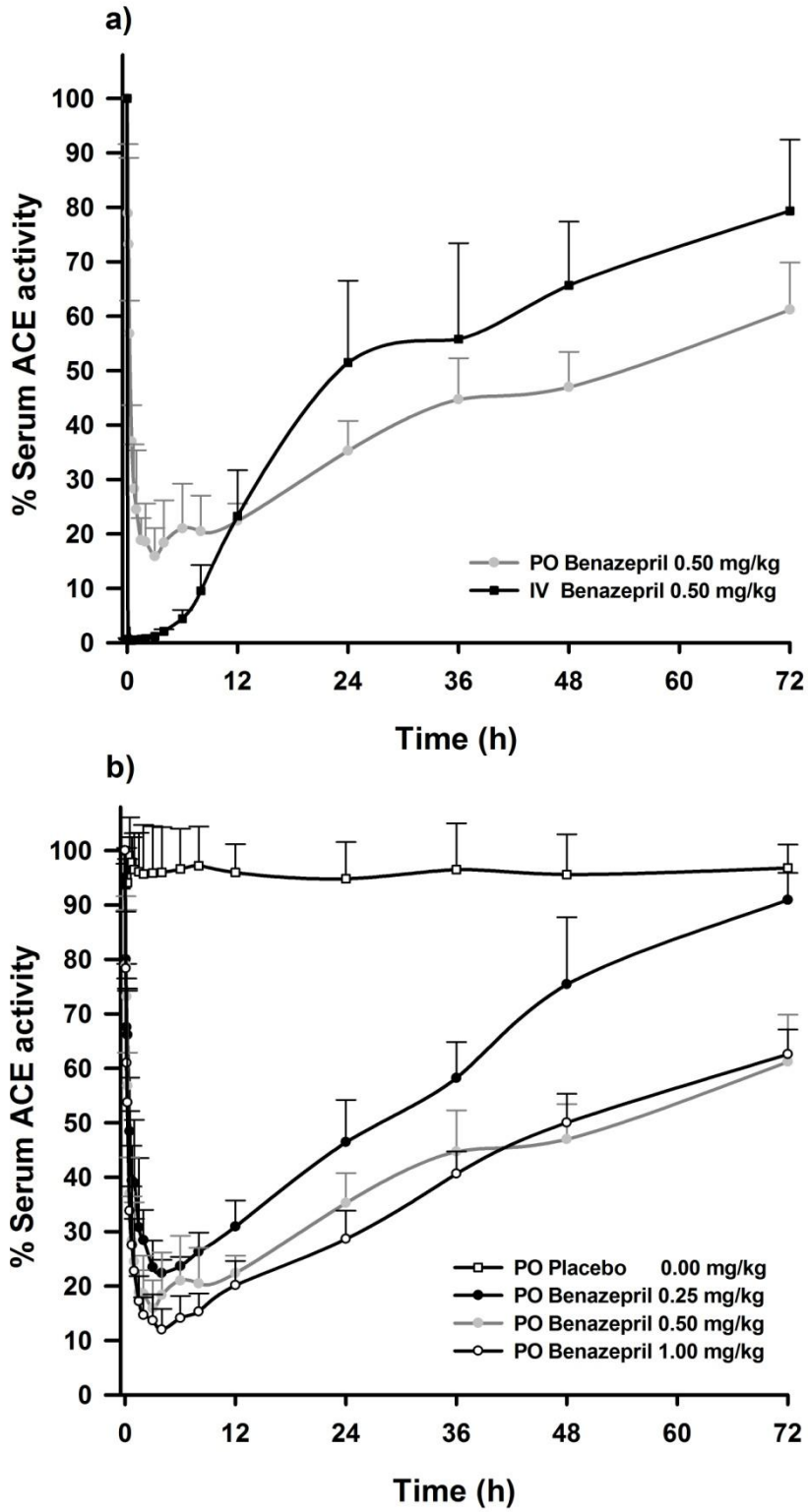

Fig. 2

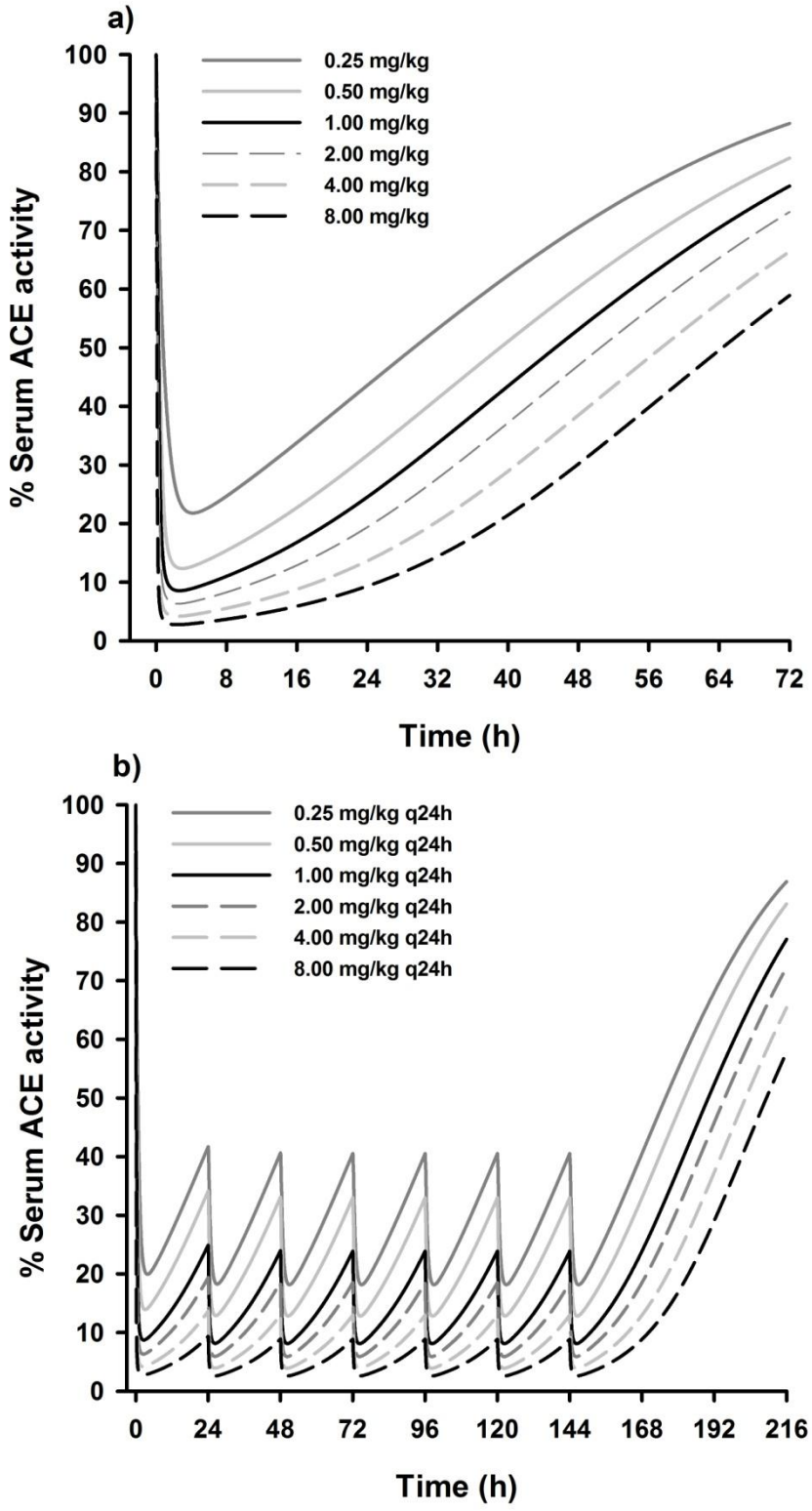

Fig. 3

Table 1

Pharmacokinetic-pharmacodynamic parameters (median with interquartile ranges) for benazepril and benazeprilat after different PO and IV benazepril doses.

	Doses and routes			
Parameters	0.50 mg/kg (IV)	0.25mg/kg (PO)	0.50 mg/kg (PO)	1.00 mg/kg (PO)
Benazepril				
k_a (1/h)		0.09 (0.03)	0.07 (0.05)	0.08 (0.03)
k_f (1/h)	7.47 (0.93)	6.53 (0.51)	6.86 (0.38)	5.80 (0.47)
Benazeprilat				
V_c (L/kg)	0.25 (0.05)			
V_c/F (L/kg)		4.45 (0.37)	4.26 (0.62)	4.72 (1.00)
Cl (L/h/kg)	0.29 (0.06)			
Cl/F (L/h/kg)		6.47 (1.56)	7.87 (1.65)	6.57 (1.55)
F (%)		4.29 (1.19)	3.28 (1.74)	4.30 (1.39)
k_{10} (1/h)	1.17 (0.30)	1.50 (0.49)	1.88 (0.53)	1.36 (0.52)
$t_{1/2k10}$ (h)	0.60 (0.16)	0.46 (0.13)	0.37 (0.12)	0.51 (0.20)
K_d (nmol/l)	1.91 (0.21)	4.22 (4.40)	1.22 (1.75)[a]	1.22 (0.84)[a]
B_{max} (nmol/l)	107.74 (68.98)	96.98 (59.60)	84.18 (12.73)	144.04 (101.30)[b]
f_{circ} (%)	16.55 (11.15)	18.68 (3.55)	14.48 (3.73)	10.72 (8.26)[b]
P_{max} (nmol/l)	13.31 (23.63)	18.56 (14.57)	12.16 (4.86)	15.74 (27.40)[b]
IC_{50} (nmol/l)	0.55 (0.31)	0.81 (0.09)	0.80 (0.07)	1.12 (0.17)
γ	0.60 (0.03)	0.65 (0.08)	0.58 (0.04)	0.61 (0.15)

k_a, absorption rate constant of benazepril; k_f,elimination rate constant of benazepril by formation of benazeprilat; V_c, apparent volume of distribution for benazepril and benazeprilat; V_c/F, apparent volume of distribution for benazepril and benazeprilat with respect to the bioavailability; Cl, clearance of free benazeprilatafter IV benazepril; Cl/F, clearance of free benazeprilat with respect to the bioavailability; F, PO bioavailability of benazeprilat after IV benazepril; k_{10}, elimination rate constant of free benazeprilat; $t_{1/2k10}$, half-life of elimination of free benazeprilat; K_d,equilibrium dissociation constant of free benazeprilat producing saturation of 50% of ACE; B_{max}, total binding capacity of the ACE; f_{circ}, fraction between circulating and noncirculating ACE; P_{max}, total binding capacity of the circulating ACE; IC_{50}, free benazeprilat concentration required to produce 50% of ACE inhibition; γ, Hill coefficient which describes the steepness of the concentration effect curve.

[a]Significantly different from 0.50 mg/kg PO dose ($p<0.05$).

[b]Significantly different from 0.25 and 0.50 mg/kg PO doses ($p<0.05$).

Table 2.

Serum ACE inhibitions (median with interquartile ranges) after different IV and PO benazepril doses.

	Doses and routes				
Dose	0.50 mg/kg	0.00 mg/kg	0.25 mg/kg	0.50 mg/kg	1.00 mg/kg
Route	IV	PO (placebo)	PO	PO	PO
$\%I_{max}$	99.63 (0.12)	6.77 (8.67)	78.91 (2.64)	85.74 (8.31)[a,c]	89.51 (6.70)[a]
$T_{\%Imax}$(h)	0.75 (0.63)	2.50 (3.25)	3.50 (1.00)[b]	3.00 (1.00)[b,c]	4.00 (2.25)[b]
$\%I_{12h}$	73.93 (13.03)	4.74 (3.20)	69.99 (10.41)[b]	77.97 (5.32)[a,c]	80.58 (8.70)[a]
$\%I_{24h}$	46.54 (22.69)	3.60 (9.04)	52.52 (20.03)[b]	64.56 (6.11)[a,c]	70.80 (8.58)[a]
$\%I_{48h}$	34.66 (22.59)	9.14 (4.54)	24.50 (29.95)[b]	51.98 (10.40)[a,c]	51.69 (9.15)[a]
$\%I_{72h}$	16.61 (19.19)	4.27 (3.02)	2.11 (6.21)	40.08 (14.13)[a,c]	38.84 (8.64)[a]

$\%I_{max}$, maximum serum ACE inhibition from 0 to 12 h; $T_{\%Imax}$, time to reach the $\%I_{max}$ value; $\%I_{12h}$, maximum serum ACE inhibition at 12 h;$\%I_{24h}$, maximum serum ACE inhibition at 24 h; $\%I_{48h}$, maximum serum ACE inhibition at 48 h; $\%I_{72h}$, maximum serum ACE inhibition at 72 h.

[a]Significantly different from 0.00 mg/kg (placebo) and 0.25 mg/kg PO dose ($p<0.05$).

[b]Significantly different from 0.00 mg/kg (placebo) ($p<0.05$).

[c]Significantly different from 0.50 mg/kg IV dose ($p<0.05$).

Figure lengends

Fig. 1. (a) Semilogarithmic plot of serum concentrations (mean ± SD) of benazepril (-■-) and benazeprilat (-□-) after IV benazepril at 0.50 mg/kg, and benazepril (-■-) and benazeprilat (-□-) after PO benazepril at 0.50 mg/kg in horses. (b) Semilogarithmic plot of serum concentrations (mean ± SD) of benazepril (-●-,-■-, -♦-) and benazeprilat (-○-,-□-,-◊-) after PO benazepril at 0.25, 0.50 and 1.00 mg/kg in horses, respectively.

Fig. 2. (a) Mean serum ACE activity expressed as percent of control after IV benazepril at 0.50 mg/kg (-■-) and PO benazepril at 0.50 mg/kg (-●-). (b) Mean serum ACE activity expressed as percent of control after PO doses of placebo (0.00 mg/kg, -□-), of benazepril at 0.25 (-●-), at 0.50 (-●-) and at 1.00 mg/kg (-○-), respectively.

Fig. 3. (a) Plot of predicted effects on serum ACE activity in horses after single PO doses of benazepril from 0.25 to 8.00 mg/kg. (b) Plot of predicted effects on serum ACE activity in horses after multiple PO benazepril from 0.25 to 8.00 mg/kg for a week.

Highlights

Angiotensin converting enzyme inhibitor benazeprilat was evaluated in horses after different IV and PO doses of benazepril.

Pharmacokinetics and pharmacodynamics relationships were obtained and investigated.

Oral bioavailability of benazeprilat after PO benazepril was low.

Oral benazepril doses between 0.50 and 1.00 mg/kg had sufficient absorption and bioconversion to benazeprilat to induce serum ACE inhibitions near to 88%.

Further works with different doses or formulations in equine patients should be investigated.