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Pharmacodynamic Evaluation of 4 Angiotensin-Converting Enzyme Inhibitors in Healthy Adult Horses

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Background: Angiotensin-converting enzyme (ACE) inhibitors are used in horses with cardiovascular disorders despite the paucity of available data regarding their efficacy.

Hypothesis: The degree of serum ACE inhibition varies considerably between drugs.

Animals: Eight healthy adult horses.

Methods: Randomized prospective study. Horses were fasted overnight prior to receiving one of 4 ACE inhibitors intragastrically, administered at one of 2 dosages, using a randomized Latin square design (benazepril: 0.5 and 0.25 mg/kg; ramipril: 0.3 and 0.1 mg/kg; quinapril: 0.25 and 0.125 mg/kg; perindopril: 0.1 and 0.05 mg/kg). Serum ACE activity was measured using a kinetic spectrophotometric method.

Results: There was a significant effect of drug and dosage on maximum ACE inhibition (I_{max}), ACE inhibition 24 hours after administration (I_{24h}), and area under the curve ($AUC_{0–48h}$). Benazepril at 0.5 mg/kg resulted in significantly higher I_{max} (86.9 ± 7.0%) and I_{24h} (60.3 ± 7.9%) compared to the other drugs. There was a significant decrease in indirect blood pressure (BP) over time after administration of each drug, but differences in BP were not significantly different between drugs. Pharmacodynamic variables measured after administration of benazepril to horses with free access to hay were not significantly different from those obtained after fasting. Administration of benazepril orally once daily for 7 days did not result in a cumulative effect on ACE inhibition.

Conclusions and Clinical Importance: Of the ACE inhibitors tested, oral benazepril (0.5 mg/kg) is the most effective at inhibiting serum ACE activity in healthy horses.

Key words: ACE inhibitor; Benazepril; Cardiology; Equine; Perindopril; Quinapril; Ramipril.

The use of angiotensin-converting enzyme (ACE) inhibitors represents a major advance in the medical management of cardiovascular diseases in people, dogs, and cats.[1,2] In dogs with congestive heart failure (CHF) caused by myxomatous mitral valve disease or dilated cardiomyopathy, ACE inhibitors have beneficial hemodynamic and clinical effects, improving quality of life and extending survival times by 2- to 3-fold.[1] Other indications for the use of ACE inhibitors include treatment of systemic hypertension and chronic renal failure.[3] ACE inhibitors approved for use in dogs and cats include benazepril, enalapril, imidapril, and ramipril.[1] Several other ACE inhibitors are approved for use in people; of these, perindopril offers the advantage of having high lipophilicity, leading to enhanced tissue penetration and higher binding affinity.[4,5]

Disease processes such as myxomatous degenerative valve disease, congenital cardiac defects, aorto-cardiac fistulas, and myocardial dysfunction have been reported to cause CHF in horses, with degenerative mitral valve disease being the most common.[6] Activation of the renin-angiotensin-aldosterone system also occurs in horses with valvular insufficiency,[7] the maladaptive effects of which have been well-described in other species and include sodium and fluid retention, vasoconstriction, and tissue fibrosis. The harm imposed by these effects in the setting of severe heart disease has led to the hypothesis that the use of ACE inhibitors would provide therapeutic benefit. As a result, ACE inhibitors have been recommended widely for the treatment of severe valvular diseases and CHF in horses despite the paucity of available data regarding their efficacy.

Abbreviations:

ACE	angiotensin-converting enzyme
BP	blood pressure
CHF	congestive heart failure

The ACE inhibitor enalapril has been widely recommended in horses.[8,9] However, enalapril has very low oral bioavailability and does not inhibit ACE in horses when given via this route.[10,11] Other ACE inhibitors investigated in horses include quinapril and ramipril. In horses with mitral insufficiency, oral administration of quinapril at a dose of 120 mg/day increased stroke volume and cardiac output, and decreased mitral regurgitation velocity time integral.[12] However, the study lacked an untreated control group; therefore, it is difficult to differentiate a true effect of quinapril from that of changes in hemodynamics between time points. Oral administration of ramipril at 0.06 mg/kg attenuated the rise in blood pressure (BP) caused by exogenous angiotensin I[13] and oral administration of ramipril at 0.2 mg/kg to healthy horses resulted in a significant decrease in BP.[14]

The lack of data regarding the relative potency of PO administered ACE inhibitors is a major problem

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that prevents the rational use of these agents in horses with CHF. Therefore, the main objective of this study was to compare the relative potency of benazepril, quinapril, perindopril, and ramipril in terms of each drug's ability to inhibit serum ACE activity in horses. We hypothesized that the degree of ACE inhibition would vary considerably between drugs.

Materials and Methods

Animals

Eight nonpregnant mares ranging from 13 to 20 years of age and weighing between 515 and 644 kg were selected for this study. Represented breeds included Thoroughbreds (n = 4), Quarter Horse (1), Appaloosa (1), Trakehner (1), and Saddlebred-cross (1). The animals were deemed clinically healthy based on the results of a thorough physical examination, complete blood count, and plasma biochemical profile. The study was approved by the Institutional Animal Care and Use Committee of the University of Georgia.

Comparative Pharmacodynamics of 4 ACE Inhibitors after a Single Intragastric Dose in Horses

Four ACE inhibitors (quinapril HCL [MW: 474.98 g/mol],[a] ramipril [MW: 416.51 g/mol],[b] benazepril HCL [MW: 460.96 g/mol],[c] and perindopril erbumine [MW: 446.61.98 g/mol][d]) were studied. Each drug was given at a low and a high dose (benazepril: 0.25 and 0.5 mg/kg; perindopril: 0.05 and 0.1 mg/kg; quinapril: 0.125 and 0.25 mg/kg; ramipril: 0.1 and 0.3 mg/kg) using a balanced randomized Latin square design. Dosages were extrapolated from the low and high ends of the dosage regimens recommended for people (perindopril),[19] dogs (benazepril, ramipril),[1] and horses (quinapril).[12,e] There was a washout period of 1 week between each administration. Horses were placed in a stall at approximately 16 hours before drug administration to allow for acclimatization and remained in the same stall for 8 hours after drug administration. Horses were fasted overnight before administration of each drug. Tablets were dissolved in water, suspended in approximately 350 mL of water, and administered by nasogastric intubation. Feed was offered 2 hours after drug administration. Horses had ad libitum access to water at all times.

Blood samples for the measurement of ACE activity were collected by jugular venipuncture before (time zero) and at 0.5, 1, 2, 4, 8, 24, and 48 hours after drug administration. Blood samples were incubated at 37°C for 15 minutes, centrifuged at 1,500 × *g* for 10 minutes, and serum was stored at −20°C until assayed. Heart rate, systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP) were obtained before (time zero) and 1.5, 4, and 8 hours after drug administration using an oscillometric device[f] with a cuff with a bladder width approximating 30–40% of the measurement site circumference placed at the base of the tail over the coccygeal artery. The head of each horse was maintained in a constant position during BP measurement. Measurements were obtained in duplicate and the mean of the 2 measurements was used for data analysis. When the difference in MAP between the duplicate measurements was greater than 10%, a third measurement was taken and the median of the 3 measurements was used for data analysis. For each horse, drug, and dose combination, the lowest BP measurement, and time to lowest BP measurement were determined.

Effect of Feeding on the Pharmacodynamics of Benazepril

After a washout period of 2 weeks, benazepril was given at a dose of 0.5 mg/kg by nasogastric intubation to the same 8 horses. Drug administration was performed exactly as described above, except that horses had ad libitum access to grass hay and water before and after administration of the drug. Blood samples for measurement of ACE activity were collected as described above. Heart rate and BP were not measured.

Pharmacodynamics of Benazepril after Repeated Oral Administrations

Six healthy nonpregnant mares were used in this experiment. Low (0.25 mg/kg) or high (0.5 mg/kg) dose benazepril was administered PO once a day for 7 days using a cross-over design with a 1-week washout between treatment periods. The tablets were dissolved and suspended in 20 mL of water, mixed with approximately 25 mL of molasses, and administered PO using a 60 mL dose syringe to simulate a field situation. Blood samples were collected by jugular venipuncture before (time zero) and 0.5, 1, 2, 4, 8, and 24 hours after drug administration on days 1 and 7. On days 3–6, a single blood sample was collected before administration of the drug. Horses were kept on pasture with free access to grass and water, except for the 8 hours of frequent sample collection on days 1 and 7, during which they were in a stall with access to grass hay and water.

Measurement of ACE Inhibition

Angiotensin-converting enzyme activity was measured using a modification of a commercially available kinetic spectrophotometric method,[g,h] that has been used successfully to measure ACE activity in horses.[16] This method is based on the fact that hydrolysis of the substrate (N-[3-(2-furyl)-acryloyl]-L-phenylalanylglyclglycine) by ACE at 37°C results in a decrease in absorbance at 340 nm. The 2-fold dilutions of a high concentration positive control[g] were used to generate a standard curve ranging from 120 to 1.875 U/L on each plate to account for potential variation between plates. Standards and serum samples (30 μL each) were added to a 96-well plate before the addition of 300 μL of reagents. The plates were incubated at 37°C. Absorbance at 340 nm was measured after 5 and 30 minutes of incubation using a microplate reader.[i] The lower limit of quantification of the assay was 3.75 U/L, and the lower limit of detection was 1.875 U/L. Plots of the difference in absorbance (5–30 minutes) versus known ACE activity of the standards were linear on each day of analysis ($R^2 = 0.998 \pm 0.002$). Both intraday and interday coefficients of variation for repeatedly assayed samples were <9%. For each horse, drug, and dose combination, the results were expressed as percentage ACE inhibition relative to the respective baseline value.

Pharmacodynamic Analysis

For each horse, serum ACE inhibition versus time data was analyzed based on a noncompartmental approach using computer software.[j] The rate constant of the terminal phase (λ_z) was determined by linear regression of the logarithmic serum ACE inhibition versus time curve using a minimum of three data points. Half-life of the terminal phase ($t_{1/2\lambda z}$) was calculated as the natural logarithm of two divided by λ_z. The area under the concentration-time curve (AUC) was calculated using the trapezoidal rule, with extrapolation to infinity using I_{min}/λ_z, where I_{min} was the final measurable ACE inhibition. Additional pharmacodynamic

parameters calculated included maximum ACE inhibition (I_{max}), time to maximum ACE inhibition (T_{max}), ACE inhibition at 24 hours (I_{24h}), and duration of ACE inhibition ($T_{baseline}$).

Statistical Analyses

Normality and equality of variance of the data were assessed using Shapiro–Wilk's and Levene's tests, respectively. Preliminary data analysis was conducted using a one-way ANOVA with repeated measures to ensure that there was not a significant difference in baseline ACE activity throughout the study. A general linear model with repeated measures was used to assess the effect of drug, dose (high versus low), and interactions between drug and dose on each pharmacodynamic parameter. A similar model was used to analyze heart rate and indirect BP data. A paired *t*-test was used to compare the pharmacodynamic variables of benazepril between fasted and fed horses. Finally, a general linear model with repeated measures was used to assess the effect of day of administration (1st versus 7th), dose (high versus low), and interactions between day of administration and dose on each pharmacodynamic parameter after repeated benazepril administration. When necessary, multiple pairwise comparisons were performed using the method of Holm–Sidak. For all analyses, value of $P < .05$ was considered significant. The software used was SigmaPlot 11 (Systat Software Inc, San Jose, CA).

Results

No adverse effects were observed throughout the entire study. Mean (±SD) baseline ACE activity was 59.0 ± 13.4 U/L (range 40.3–102 U/L). There was a significant effect of drug ($P < .001$) and dose ($P = .004$) on I_{max}. I_{max} was significantly higher for benazepril followed by ramipril, quinapril, and perindopril (Table 1, Fig 1). There was a significant effect of drug and dose on I_{24h} ($P < .001$ and $P = .01$, respectively) and $AUC_{0–48h}$ ($P < .001$ and $P = .006$, respectively). I_{24h} and $AUC_{0–48h}$ were significantly higher for benazepril than for all other drugs tested. Ramipril had a significantly higher I_{max} and $AUC_{0–48h}$ than quinapril and perindopril. For each drug, I_{max}, $AUC_{0–48h}$, and I_{24h} were significantly higher after administration of the high dose than after administration of the low dose (Table 1). There was a significant effect of drug but not of dose on T_{max} ($P = .002$), $AUC_{0–\infty}$ ($P < .001$), $t_{1/2\lambda z}$ ($P = .009$), and $T_{baseline}$ ($P < .001$) (Table 1).

There was a significant effect of time ($P < .001$), but no significant effect of drug ($P > .815$) and no significant interactions between time and drug ($P > .365$) on SAP, DAP, and MAP. SAP, DAP, and MAP were significantly lower at 4 and 8 hours after drug administration compared to baseline (Fig 2). SAP and MAP were also significantly lower at 1–2 hours after drug administration compared to baseline (Fig 2). Lowest BP recorded and time to lowest BP were not significantly different between drugs. The effects of time, drug, and interactions between drug and time on heart rate were not statistically significant.

Table 1. Pharmacodynamic variables after the administration of 4 ACE inhibitors (benazepril, quinapril, perindopril, and ramipril) to 8 horses. Each ACE inhibitor was given at a low and a high dose. Each horse received a single dose of one of the 8 possible drug/dose combinations using a randomized Latin square design. Data are presented as mean ± SD unless otherwise specified.

		Drug			
Variable	Dose	Quinapril	Benazepril	Ramipril	Perindopril
I_{max} (%)	H	$40.6 \pm 12.0^{a,1}$	$86.9 \pm 7.0^{b,1}$	$59.7 \pm 9.3^{c,1}$	$25.2 \pm 7.4^{d,1}$
	L	$29.6 \pm 10.3^{a,2}$	$72.2 \pm 7.5^{b,2}$	$38.2 \pm 7.0^{c,2}$	$19.9 \pm 11.8^{d,2}$
T_{max} (hours)*	H	0.5 (0.5–1)^{a,1}	2.0 (1–2)^{b,1}	0.5 (0.5–1)^{a,1}	2 (0.5–24)^{b,1}
	L	1.5 (0.5–2)^{a,1}	1.5 (0.5–8)^{b,1}	1 (0.5–8)^{a,1}	1 (0.5–2)^{b,1}
$AUC_{0–48h}$ (%·hours)	H	$704 \pm 274^{a,1}$	$2{,}611 \pm 277^{b,1}$	$1{,}329 \pm 274^{c,1}$	$506 \pm 398^{a,1}$
	L	$421 \pm 245^{a,2}$	$2{,}217 \pm 275^{b,2}$	$1{,}086 \pm 331^{c,2}$	$377 \pm 362^{a,2}$
$AUC_{0–\infty}$ (%·hours)	H	$844 \pm 349^{a,1}$	$3{,}099 \pm 617^{b,1}$	$1{,}583 \pm 446^{b,1}$	$776 \pm 784^{a,1}$
	L	$426 \pm 257^{a,1}$	$2{,}723 \pm 495^{b,1}$	$1{,}571 \pm 674^{b,1}$	$778 \pm 1{,}192^{a,1}$
$t_{½\lambda z}$ (hours)	H	$15.3 \pm 10.6^{a,1}$	$17.5 \pm 7.6^{a,b,1}$	$17.5 \pm 6.8^{b,1}$	$22.5 \pm 18.5^{a,b,1}$
	L	$10.0 \pm 5.0^{a,1}$	$20.3 \pm 3.5^{a,b,1}$	$29.8 \pm 9.2^{b,1}$	$24.8 \pm 32.8^{a,b,1}$
I_{24h}	H	$14.2 \pm 8.4^{a,1}$	$60.3 \pm 7.9^{b,1}$	$28.7 \pm 7.2^{c,1}$	$9.6 \pm 11.5^{a,1}$
	L	$7.1 \pm 6.8^{a,2}$	$51.7 \pm 7.7^{b,2}$	$24.4 \pm 6.7^{c,2}$	$5.9 \pm 6.5^{a,2}$
$T_{baseline}$ (hours)*	H	>48 (8 to >48)^{a,1}	>48 (48 to >48)^{b,1}	>48 (>48 to >48)^{b,1}	>48 (24 to >48)^{a,1}
	L	48 (24 to >48)^{a,1}	>48 (>48 to >48)^{b,1}	>48 (48 to >48)^{b,1}	48 (2 to >48)^{a,1}

Benazepril H = 0.5 mg/kg; Benazepril L = 0.25 mg/kg; Ramipril H = 0.3 mg/kg; Ramipril L = 0.1 mg/kg; Quinapril H = 0.25 mg/kg; Quinapril L = 0.125 mg/kg; Perindopril H = 0.1 mg/kg; Perindopril L = 0.05 mg/kg.

ACE, angiotensin-converting enzyme; I_{max}, maximum ACE inhibition; T_{max}, time to maximum ACE inhibition; $AUC_{0–48h}$, area under the curve from 0 to 48 hours; $AUC_{0–\infty}$, area under the curve extrapolated to infinity; $t_{½\lambda z}$, half-life of the terminal phase; I_{24h}, ACE inhibition at 24 hours; $T_{baseline}$, time to reach baseline; H, high dose; L, low dose.

For a given dose (ie within a row), different superscript letters between drugs (quinapril versus benazepril versus ramipril versus perindopril) indicate a statistically significant ($P < .05$) difference. For a given drug, different superscript numbers indicate statistically significant differences ($P < .05$) between the 2 doses (H versus L).

*Median and range.

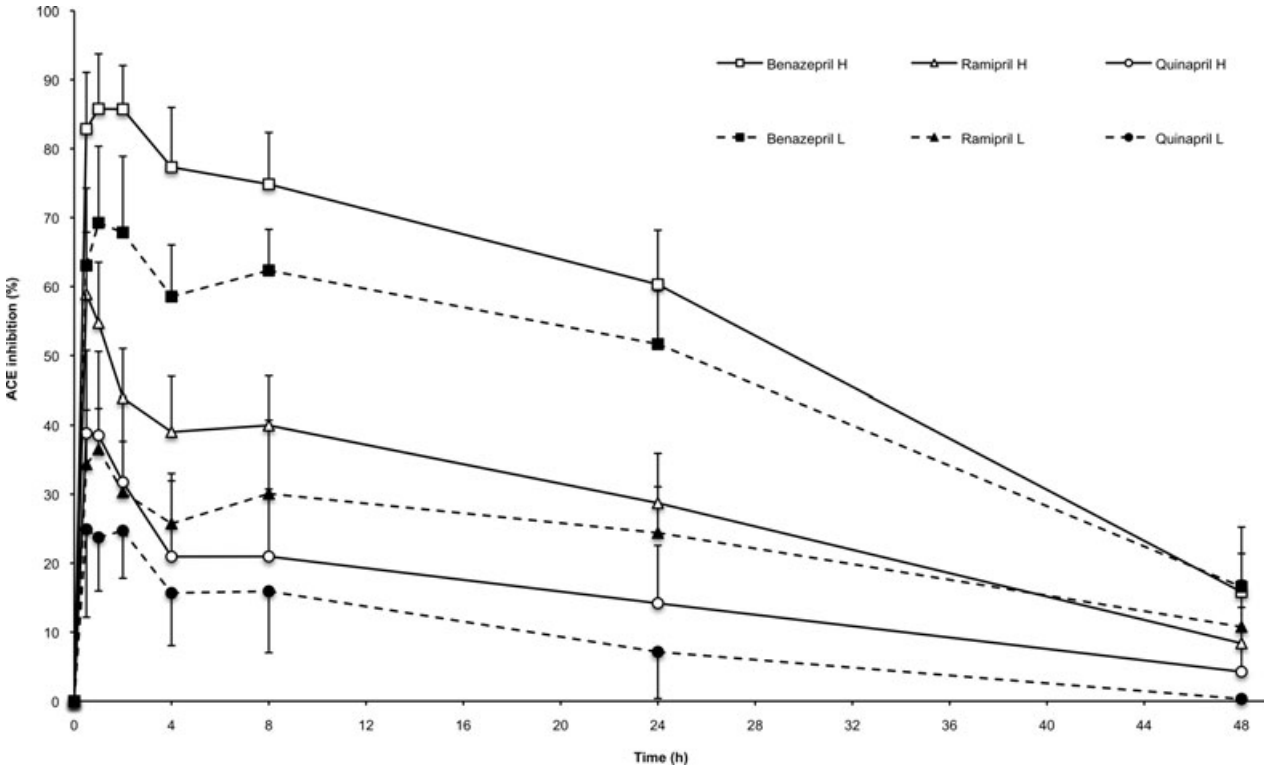

Fig 1. Mean (±SD) serum angiotensin-converting enzyme inhibition (%) in 8 horses after a single oral dose of benazepril, ramipril, or quinapril. Each drug was administered at a high (H) or low (L) dose. Benazepril H (0.5 mg/kg); Benazepril L (0.25 mg/kg); Ramipril H (0.3 mg/kg); Ramipril L (0.1 mg/kg); Quinapril H (0.25 mg/kg); Quinapril L (0.125 mg/kg).

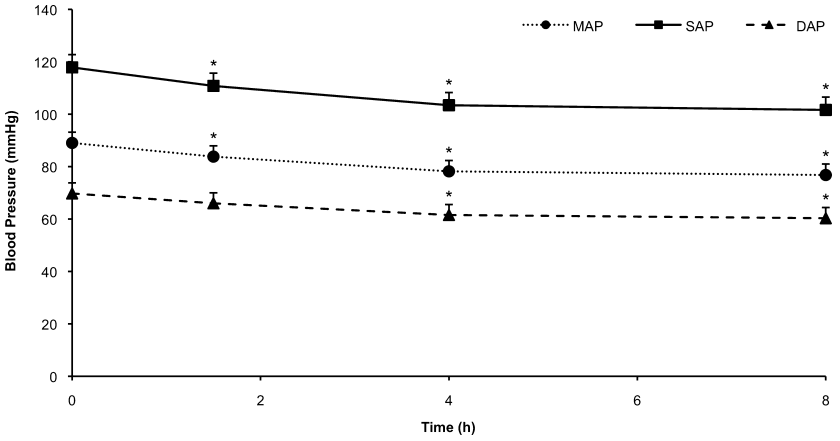

Fig 2. Least square means (±SD) of the overall effect of time on noninvasive blood pressure data after the administration of angiotensin-converting enzyme inhibitors in 8 horses given a single oral dose of quinapril, benazepril, ramipril, or perindopril. *Indicates a statistically significant difference from baseline.

Effect of Feeding on the Pharmacodynamics of Benazepril

Pharmacodynamic variables for benazepril after intragastric administration of a dose of 0.5 mg/kg to horses with ad libitum access to hay were not significantly different from those obtained after intragastric administration of the same dose to horses that were fasted (Table 2).

Pharmacodynamics of Benazepril after Repeated Administration

To investigate if multiple daily doses of benazepril would result in a cumulative effect on ACE activity, 6 horses received benazepril (0.25 or 0.5 mg/kg) PO once daily for 7 days. Multiple doses of benazepril did not result in a cumulative effect on ACE activity (Fig 3). Although there was no significant difference in I_{max}

Table 2. Pharmacodynamic variables in 8 horses administered a single dose of benazepril at a dose of 0.5 mg/kg by nasogastric intubation. Horses were either fasted or had ad libitum access of grass hay. Data are presented as mean ± SD unless otherwise specified.

Variable	Fasted	Fed	*P* value
I_{max} (%)	86.9 ± 7.0	87.3 ± 4.2	.877
T_{max} (hours)*	2.0 (1–2)	1 (0.5–2)	.125
AUC_{0-48h} (%·hours)	2,611 ± 277	2,466 ± 231	.217
$AUC_{0-\infty}$ (%·hours)	3,099 ± 617	2,977 ± 507	.689
$t_{½\lambda z}$ (hours)	17.5 ± 7.6	18.6 ± 6.2	.765
I_{24h} (%)	60.3 ± 7.9	54.0 ± 5.4	.064
$T_{baseline}$ (hours)*	>48 (48 to >48)	> 48 (>48)	1.00

I_{max}, maximum ACE inhibition; T_{max}, time to maximal ACE inhibition; AUC_{0-48h}, area under the curve from 0 to 48 hours; $AUC_{0-\infty}$, area under the curve extrapolated to infinity; $t_{½\ \lambda z}$, terminal half-life; I_{24h}, ACE inhibition at 24 hours; $T_{baseline}$, time to reach baseline.

*Median and range.

between day 1 and day 7, there was a significant decrease in T_{max}, AUC_{0-24h}, and I_{24h} from the first to the last dose (Table 3). Administration of a high dose of benazepril resulted in significantly higher I_{max} and AUC_{0-24h} compared to administration of a low dose (Table 3).

Discussion

The present study documented considerable variation in the relative potency of PO administered benazepril, perindopril, quinapril, and ramipril in horses. Of the 4 drugs tested, benazepril at a dose of 0.5 mg/kg was the most effective at inhibiting serum ACE activity. Although inhibition of circulating ACE activity is a commonly used surrogate marker to investigate the activity of ACE inhibitors, circulating ACE only represents approximately 5–30% of the total body ACE pool, depending on the species under investigation.[17] Therefore, measurement of circulating ACE activity might not accurately reflect the effects of these drugs on the larger tissular ACE pool.[15,18] Furthermore, measurement of plasma or serum ACE activity does not account for other potential beneficial effects of these drugs. In addition to blocking the conversion of angiotensin I into angiotensin II, ACE inhibitors also inhibit kininase II and increase bradykinin concentration which in turn leads to the release of nitric oxide and vasoactive prostaglandins.[3,17] In patients with CHF, the venous and arterial vasodilatory effects of ACE inhibitors ultimately provide relief of pulmonary congestion, decrease peripheral vascular resistance, increase cardiac output, and reduce hypertrophy.[19] The ultimate pharmacodynamic outcome, which should be examined to assess the relative efficacy of ACE inhibitors, would ideally be an improvement in the quality of life and in survival time of the patient with CHF. Nevertheless, measurement of circulating ACE activity, as performed in this study, has been used as a surrogate marker to assess the efficacy of these drugs.[15,18] Healthy people receiving various ACE inhibitors have BP response to exogenously administered angiotensin I that is closely correlated with circulating ACE activity.[20]

In healthy people, circulating ACE activity must be reduced by approximately 90% of baseline values to completely abolish BP increase in response to the administration of exogenous angiotensin I.[20–22] However, the minimum circulating ACE inhibition that is required to improve the quality of life and survival in veterinary or human patients with CHF is ill defined.

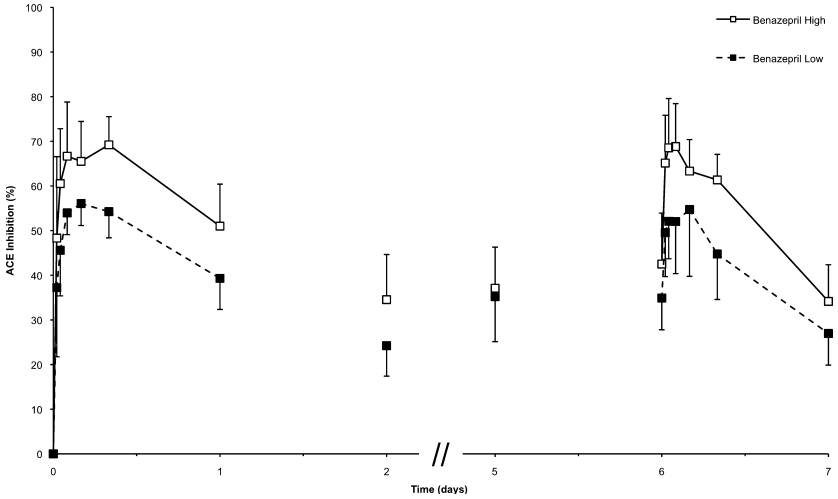

Fig 3. Mean (±SD) serum angiotensin-converting enzyme inhibition (%) in 6 horses after oral administration of benazepril at 24-h intervals for 7 days. Benazepril high = 0.5 mg/kg; Benazepril low = 0.25 mg/kg.

Table 3. Pharmacodynamic variables of benazepril after oral administration at doses 0.5 mg/kg (H) and 0.25 mg/kg (L) for 7 days. Data presented as mean ± SD unless otherwise specified.

Variable	Dose	Day 1	Day 7
I_{max} (%)	H	74.1 ± 6.5[a,1]	72.3 ± 8.5[a,1]
	L	57.7 ± 4.9[a,2]	59.9 ± 10.2[a,2]
T_{max} (hours)*	H	8 (2–8)[a,1]	2 (0.5–8)[b,1]
	L	6 (1–8)[a,1]	1.5 (0.5–4)[b,1]
AUC_{0-24h} (%·hours)	H	1,465 ± 146[a,1]	1,275 ± 85.8[b,1]
	L	1,159 ± 101[a,2]	978 ± 187[b,2]
I_{24h} (%)	H	51.0 ± 9.4[a,1]	34.1 ± 8.2[b,1]
	L	39.3 ± 7.0[a,1]	27.0 ± 7.1[b,1]

I_{max}, maximum ACE inhibition; T_{max}, time to maximum ACE inhibition; AUC_{0-24h}, area under the curve from 0 to 24 hours; I_{24h}, ACE inhibition at 24 hours; H, High dose; L, Low dose.

For a given dose (ie within a row), different superscript letters indicate a statistically significant ($P < .05$) difference between day 1 and day 7. Within a given day, different superscript numbers indicates statistically significant differences ($P < .05$) between the 2 doses (H versus L).

*Median and range.

Oral administration of enalapril or benazepril at recommended dosages to normal dogs results in I_{max} of approximately 63 and 81%, respectively.[23] Yet, these drugs have been demonstrated to extend the life span of dogs with CHF in clinical trials,[24,25] suggesting that a 90% inhibition in circulating ACE activity might not be necessary to observe clinical benefit from their administration. Of the ACE inhibitors evaluated in the present study, only benazepril resulted in an I_{max} >60%. For each drug evaluated in the present study, ACE inhibition was significantly higher after administration of the high dose compared to the low dose. This suggests that serum inhibition might be dose-dependent. However, studies in dogs have shown that this dose-dependent effect reaches a plateau at higher doses.[26] The lack of dose proportionality of ACE inhibitors is explained by their saturable binding to ACE.[27] Additional studies would be required to determine if ACE inhibitor dosages greater than those investigated in the present study would result in a proportional increase in ACE inhibition after oral administration to horses.

In the present study, administration of oral perindopril resulted in a significantly lower I_{max} compared to the other 3 drugs studied. However, this must be interpreted with caution, because the high dose for perindopril was similar to the low dose of quinapril and ramipril. Indeed, I_{max} after administration of perindopril at 0.1 mg/kg (25.2 ± 7.4%) was similar to that achieved after administration of quinapril at 0.125 mg/kg (29.6 ± 10.3%) and ramipril at 0.1 mg/kg (38.2 ± 7.0%). Similarly, the greater activity of benazepril might be caused by the higher dose used, although I_{max} for benazepril was greater than for quinapril at equivalent doses of these drugs (0.25 mg/kg; I_{max}, 72.2 ± 7.5% and 40.6 ± 12.0%, respectively). Doses were extrapolated from the low and the high end of the dosage regimens recommended in people (perindopril),[15] dogs (benazepril, ramipril),[1] and horses (quinapril).[12,e]

Plasma concentrations of the prodrugs or their active metabolites (benazeprilat, perindoprilat, quinaprilat, and ramiprilat) were not measured in this study. As a result, it is unknown if the relative differences in serum ACE inhibition are because of the differences in oral bioavailability, differences in conversion from the prodrug to the active metabolite in the liver, differences in the ability of each drug to inhibit circulating ACE, or to a combination of these factors. In dogs and cats, plasma concentrations of ACE inhibitors are poor predictors of ACE activity.[18,27] Similarly, oral administration of quinapril to healthy adult horses resulted in a significant decrease in ACE activity despite concentrations of quinaprilat below the limit of quantification of the assay used (0.1 μg/mL).[e]

Despite marked differences in ACE inhibition between the different drugs and dosages evaluated, each drug and dose combination caused a similar reduction in indirect BP. The magnitude and timing of reduction in BP in the present study were similar to those reported in dogs, in which the maximal effect after treatment does not generally exceed a 5–10 mmHg decrease, 1–6 hours after dosing.[28] Unfortunately, BP was only measured for 8 hours after administration in the present study; thus, the duration of the significant decrease in BP could not be determined. As stated above, a decrease in systemic vascular resistance is only one of the many beneficial effects ACE inhibitor therapy. Therefore, the lack of significant differences in the reduction of BP between drugs cannot be taken as evidence of equivalent clinical efficacy. Additionally, in the normal animal, BP reduction would be expected to be a relatively insensitive indicator of drug effect, as the vasodilatory potential of these drugs is likely masked in the absence of pathologic renin-angiotensin-aldosterone system activation.

Consistent with the results of studies with various ACE inhibitors in dogs and cats,[15] feeding did not affect the pharmacodynamics of benazepril when administered intragastrically to horses in the present study. Based on a relatively long $t_{1/2\lambda z}$ (18.6 ± 6.2 hours) and relatively high I_{24} (54.0 ± 5.4%) achieved after intragastric administration of benazepril at 0.5 mg/kg to horses with ad libitum access to hay, pharmacodynamic modeling would have predicted a considerable cumulative effect of repeated daily administrations on ACE inhibition. In contrast to calculated predictions, there was a significant decrease in I_{24} and AUC_{0-24} from day 1 to day 7 of repeated daily administrations. Studies in people and dogs have shown minimal to no cumulative effects on ACE inhibition with repeated administration.[15,26,29,30] However, the significant decrease in I_{24} and AUC_{0-24} were unexpected. Similarly, the considerably lower I_{max} after oral administration of benazepril (74 ± 6.5%) when compared to that achieved after intragastic administration (87.3 ± 4.4%) was unforeseen, but may reflect loss of drug during suspension preparation or administration. Besides the method of administration (oral

versus intragastric), the only other difference in the management of the horses between the 2 studies was that the horses were on pasture with access to grass the night before oral administration, whereas they were in a stall with access to grass hay the night before intragastric administration. Thus, it is unknown if the method of administration, access to pasture, or both influenced the degree of ACE inhibition.

In conclusion, administration of benazepril resulted in significantly greater serum ACE inhibition than the administration of quinapril, ramipril, or perindopril at the doses tested. Feeding before benazepril administration does not negatively impact the pharmacodynamics of the drug. Administration of multiple doses does not result in a cumulative effect of the drug on serum ACE inhibition. Further studies will be required to assess the pharmacodynamics and clinical efficacy of benazepril in horses with volume overload or signs of CHF.

Footnotes

[a] Quinapril Hydrochloride; Greenstone LLC, Peapack, NJ
[b] Ramipril; Cobalt Laboratories, Bonita Springs, FL
[c] Benazepril Hydrochloride; Ranbaxy Pharmaceuticals Inc, Jacksonville, FL
[d] Perindopril Erbumine; Aurobindo Pharma USA, Inc, Dayton, NJ
[e] Kruger K, Davis JL, Breuhaus B. Pharmacokinetics and pharmacodynamics of quinapril in horses. J Vet Intern Med 2011;25:677 (abstract)
[f] SurgiVet® V6004 NIBP Monitor; Smiths Medical PM, Inc, Waukesha, WI
[g] Infinity™; ACE Control Set, Fisher Scientific Company, LLC, Middletown, VA
[h] Infinity™ ACE Assay; Fisher Scientific Company
[i] Synergy HT Multi-Mode Microplate Reader; BioTek Instruments, Inc, Winooski, VT
[j] PK Solutions 2.0; Summit Research Services, Montrose, CO

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