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An Angiotensin Converting Enzyme Inhibitor, Benazepril Can Be Transformed to an Active Metabolite, Benazeprilat, by the Liver of Dogs with Ascitic Pulmonary Heartworm Disease

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ABSTRACT. To examine whether an angiotensin converting enzyme (ACE) inhibitor, benazepril, can be transformed to the active metabolite, benazeprilat, by severely injured liver of dogs with ascitic heartworm disease, benazepril hydrochloride was administered orally to dogs once daily for 7 consecutive days at a dose rate of 0.29 mg/kg to 0.63 mg/kg of body weight, and plasma benazepril and benazeprilat concentrations were determined on the 1st and 7th administration days. In 7 dogs with ascitic pulmonary heartworm disease, plasma benazeprilat concentrations tended to be higher than in 7 control dogs both on the 1st and 7th administration days. The peak concentration and area under the concentration-time curve tended to be greater in dogs of the ascites group than in control dogs, but the statistics could not detect significant differences in the time to peak concentration and $t_{1/2}$ between the control and ascites groups. Plasma ACE activities decreased after administration of benazepril. In dogs with ascitic heartworm disease, benazepril was readily transformed to benazeprilat by the liver, and was effective for suppression of plasma ACE activity.

KEY WORDS: ascites, benazepril, benazeprilat, canine, heartworm disease.

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Angiotensin converting enzyme (ACE) inhibitors have been widely used for the treatment of congestive heart failure in dogs [3, 6, 11]. In dogs with heartworm disease, circulatory disturbance is induced by pulmonary hypertension [10], and the renin-angiotensin system is activated [2], thus leading to a possible exacerbation of the circulatory disturbance through vascular constriction. Therefore, the inhibition of ACE activity may be effective for the improvement of circulation through vasodilative action in dogs with heartworm disease.

In heartworm disease, ascites is recognized as the most severe and terminal indication, and results from severe circulatory disturbance and liver injury [12, 17]. Long acting ACE inhibitors (enalapril and benazepril) are pro-drugs, and are transformed to active metabolites (enalaprilat and benazeprilat) in the liver, which act as potent ACE inhibitors [14]. As reported in human patients with cirrhosis [13], the conversion from pro-drug to an active metabolite may also be reduced in dogs with ascitic heartworm disease, because of liver dysfunction. Dogs with heartworm disease also displayed renal injury [19], and drug elimination was retarded in animals with renal dysfunction [15]. The objective of this study was to investigate whether benazepril can be transformed to benazeprilat in the liver of dogs with ascitic heartworm disease.

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MATERIALS AND METHODS

Dogs: Seven dogs with pulmonary heartworm disease having obvious ascites were used (ascites group, Table 1). The dogs were outpatients of the Veterinary Hospital of Gifu University. After informed consent along with a document about details of the trial (e.g., schedule, advantages, and possible adverse reactions), approvals were obtained from the owners of the dogs. As the control group, 7 heartworm-free normal adult dogs were used. Ages, sexes, and body weights of the experimental dogs are shown in Table 1. In the ascitic group, 4 dogs were male and 3 dogs were female. Body weights ranged from 7.5 kg to 17.6 kg. In the control group, all dogs were female, and body weights ranged from 5.9 kg to 19.5 kg. They received monthly administrations of a prophylactic against heartworm infection (Milbemycin oxim, Sankyo Co., Ltd., Tokyo).

Drugs: Benazepril hydrochloride tablets (Fortecor, Novartis Animal Health, Tokyo, Japan) at a dose rate of 0.29 mg/kg to 0.63 mg/kg of body weight were administered orally once daily for 1 week. A given dose of the drug was 0.25 to 0.50 mg/kg [7, 8]. Concomitant administrations of furosemide (Lasix, Hoechst Marion Roussel, Tokyo, Japan), metildigoxin (Lanirapid, Yamanouchi Pharamaceutical Co., Ltd., Tokyo, Japan), amynophillin (Neophillin, Eisai Co., Ltd., Tokyo, Japan), and isosorbide dinitrate (Frandle, Yamanouchi Pharamaceutical Co., Ltd., Tokyo, Japan) were used together with benazepril, because most of the ascitic dogs had received concomitant drugs before the trial (Table 1). Other vasodilators, including other ACE inhibitors, were not used.

Table 1. Outline of experimental dogs and drugs used in combination

Group	Dog No.	Age (year)	Sex	Breed	Body weight (kg)	Dose of benazepril (mg/kg)	Heartworm infection: Micro-filaria	Heartworm infection: Antigen	Heartworm infection: Echo-cardiography	Concomitant drugs
Ascites	4444	17	Male	Mixed	18.0	0.29	+	++	+	Furosemide (1.1 mg/kg), isosorbide dinitrate (0.56 mg/kg)
	4458	9.5	Male	Shiba dog	9.6	0.52	–	+	+	Furosemide (1.0 mg/kg)
	4480	18	Female	Mixed	8.5	0.58	–	+	+	Furosemide (1.2 mg/kg), metildigoxin (2.9 μg/kg), isosorbide dinitrate (0.59 mg/kg)
	4481	14	Male	Mixed	7.5	0.63	–	+	+	Furosemide (2.7 mg/kg), aminophyllin (3.5 mg/kg)
	4489	11	Male	Mixed	14.1	0.51	–	+	+	Furosemide (0.7 mg/kg)
	4490	8	Female	Siberian husky	17.6	0.55	–	–	+	Furosemide (3.4 mg/kg), metildigoxin (1.4 μg/kg), isosorbide dinitrate (0.57 mg/kg)
	4533	16	Female	Mixed	11.7	0.53	–	+	+	Furosemide (1.7 mg/kg), metildigoxin (2.1 μg/kg), isosorbide dinitrate (0.85 mg/kg)
Control	4367	8	Female	Siberian husky	19.5	0.51	–	–	–	No drugs
	4463	4	Female	Mixed	7.0	0.54	–	–	–	No drugs
	4465	2	Female	Mixed	7.4	0.51	–	–	–	No drugs
	4504	6	Female	Mixed	8.7	0.57	–	–	–	No drugs
	4516	7	Female	Mixed	8.5	0.58	–	–	–	No drugs
	4505	4	Female	Mixed	5.9	0.42	–	–	–	No drugs
	4529	5	Female	Mixed	10.2	0.49	–	–	–	No drugs

Heartworm infection; +: positive, –: negative.
Grades of heartworm serology; ++: strong positive, +: weak positive, –: negative.

Procedures: Clinical check-up was done carefully. Heartworm infection was diagnosed by echocardiography (EUB-115, Hitachi Medical Corporation, Tokyo, Japan), radiography, and detection of circulating adult-worm antigen (SNAP HW PF, IDEXX Laboratories, Tokyo, Japan) and microfilariae (filter concentration method). Blood samples for determinations of benazepril and benazeprilat concentrations were collected pre-administration, and at 0.5, 1, 2, 4, 8, 12 and 24 hr post-administration on the 1st and 7th administration days. RBC and WBC were counted with an automated cell counter (Celltac MEK-5155, Nihon Kohden Corporation, Tokyo, Japan), and plasma biochemicals (urea nitrogen, creatinine, alanine aminotransferase (ALT), alkaline phosphatase (ALP), sodium, potassium, chloride, total protein, and albumin) were determined by the dry-chemistry method (Dry-Chem 5500V and 800V, Fuji Photo-Film Co., Ltd., Tokyo, Japan). Plasma benazepril and benazeprilat concentrations were determined by a GC-MS method with mass selective detection [7, 16]. Plasma ACE activity was determined by the Kasahara method [5].

Statistics: Differences in the data between the control and ascites groups were tested using one-way ANOVA and post-hoc tests, and differences between before and after, and between the 1st and 7th day were determined using Wilcoxon's matched pair test [4].

RESULTS

Plasma concentrations of benazeprilat and benazepril: Compared with the data of the control group (Fig. 1A), plasma benazeprilat concentrations on the 1st day were variable in individual dogs of the ascites group (Fig. 1B). In the ascites group, plasma benazeprilat concentrations began to increase 0.5 hr after administration, and reached higher levels than the control group in 2 dogs (Nos. 4458 and 4490). Another 2 dogs (Nos. 4481 and 4489) had slightly higher concentrations than the control group, and 2 more dogs (Nos. 4444 and 4480) had almost the same concentrations as the control group. In a dog (No. 4533), plasma benazeprilat concentrations remained below the detection limit (1 *n*g/m*l*) up to 8 hr, began to increase from 12 hr, and rose to 33.7 *n*g/m*l* 24 hr after. If the data of this dog were excluded, plasma oncentrations between the control and ascites groups were significantly different at 4 and 8 hr. On the 7th administration day (Figs. 2A and 2B), plasma benazeprilat concentrations were higher than the control group in 2 dogs (Nos. 4458 and 4490). These two dogs had high concentrations of benazeprilat also on the 1st day of administration. In 1 dog (No. 4533), which showed a late increase of plasma benazeprilat concentration on the 1st day of administration, plasma benazeprilat concentration began to increase from 0.5 hr, and reached a peak concentration at 4 hr after administration on the 7th administration, then decreased. Mean plasma benazeprilat concentrations were higher in dogs of the ascites group than in dogs of the control group 8 and 12 hr after administration on the 7th day. Plasma benazepril concentrations (Table 2) showed the same tendency as plasma benazeprilat concentrations. Dogs having higher plasma benazeprilat concentrations tended to have higher plasma benazepril concentrations.

Table 3 shows the pharmacokinetic parameters of plasma concentrations of benazeprilat. The peak concentration of plasma benazeprilat tended to be higher in dogs with ascitic heartworm disease than in control dogs on both the 1st and

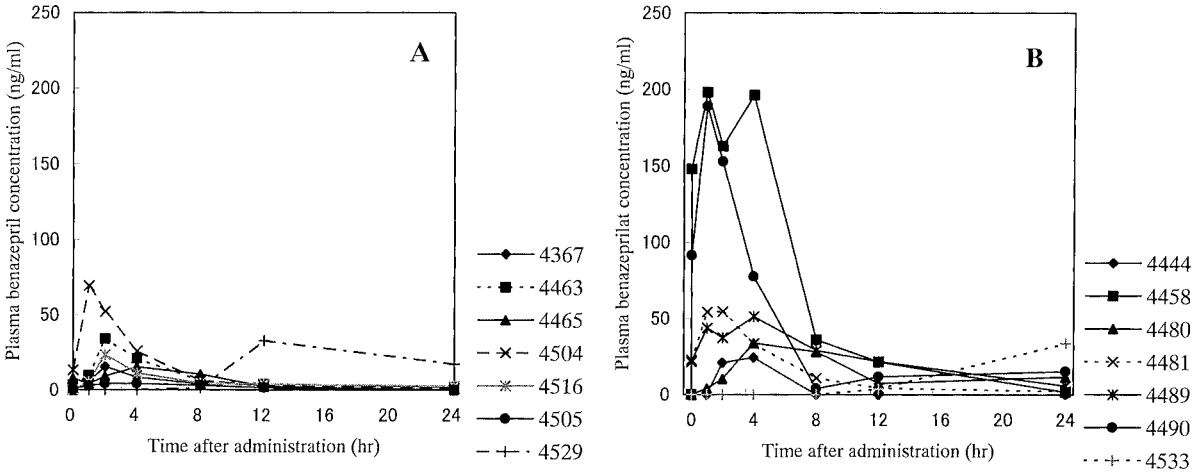

Fig. 1. Plasma benazepril concentrations on the 1st administration day in dogs of the control (A) and ascites (B) groups.

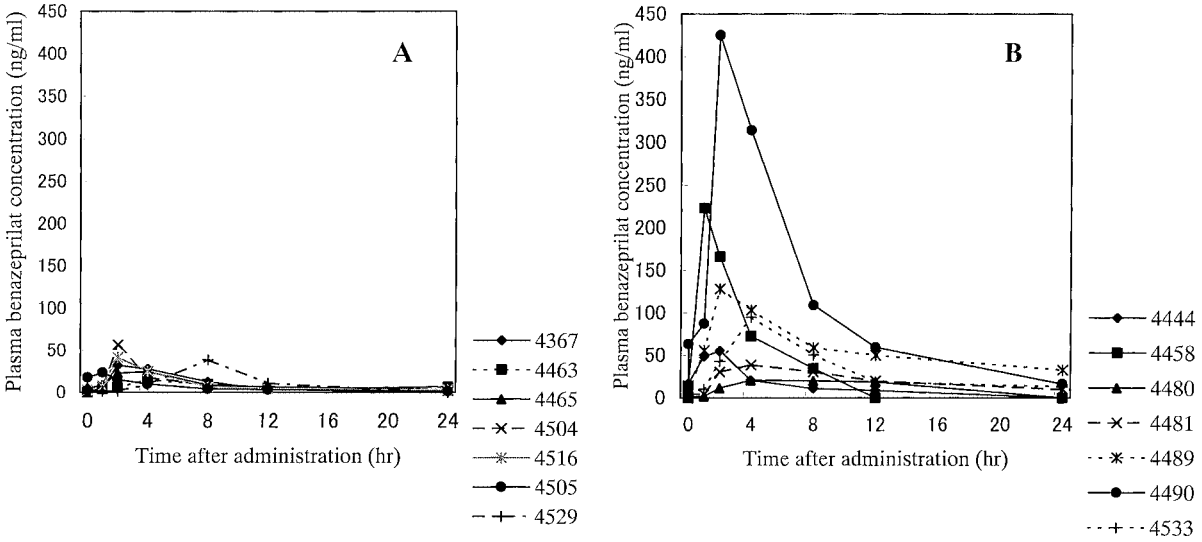

Fig. 2. Plasma benazepril concentrations on the 7th administration day in dogs of the control (A) and ascites (B) groups.

7th days. The time to peak concentration and $t_{1/2}$ were not different between the two groups on the same administration day. The area under the concentration-time curve from 0 to 24 hr ($AUC_{(0-24)}$) was higher and total body clearance was lower on the 7th day in dogs of the ascites group than in the control group. There were no significant differences in phramacokinetic parameters between the 1st and 7th day both in the control or ascites groups.

Plasma ACE activities: Plasma ACE activities are shown in Table 4. Both in dogs of the control and ascites groups, plasma ACE activity decreased after administration of benazepril on the 1st and 7th days. There were no significant differences in the plasma ACE activities measured at each point between the control and ascites groups.

Clinical signs: All 7 dogs of the ascites group showed clinical signs of severe heartworm disease such as emaciation, ascites, subcutaneous edema, exercise intolerance, coughing, labored respiration, and/or enlargement and stiffening of the liver. During benazepril administration, activity increased in one dog (No. 4481), did not change in 5 dogs, and slightly decreased in another dog (No. 4490). Other indications such as appetite, respiratory signs, exercise intolerance, and vomiting did not change during the examination period. In 7 dogs of the control group, no abnormal signs were observed during the benazepril administration. Severe pulmonary thromboembolism was observed on radiographs, and pulmonary arteries were dilated on echocardiogram, but few or no heartworm echoes were observed in the pulmonary arteries. Findings of radiography and echocardiography showed no obvious alter-

Table 2. Plasma benazepril concentrations before and 1 week after administration of benazepril

Variable	Group	Dog No.	1st day Time after administration (hours) 0	0.5	1	2	4	8	12	24	7th day Time after administration (hours) 0	0.5	1	2	4	8	12	24
Plasma benazepril concentration (*n*g/m*l*)	Control n=7	Mean	0	4.6	5.3	2.5	0	0.5	1.7	0.3	0	2.0	1.9	2.9	1.6	1.0	0	0
		SD	0	5.8	4.2	3.7	0	1.3	4.5	0.8	0	3.2	2.1	4.5	4.2	2.6	0	0
	Ascites n=7	Mean	0	41.7	12.3	2.9	2.0	0	0.2	0	0	20.4	23.3	9.4	1.3	0.9	0.4	0
		SD	0	46.6	15.9	6.9	3.7	0	0.5	0	0	30.4	22.2	9.8	2.1	1.6	1.1	0

SD: Standard deviation.

Table 3. Pharmacokinetic parameters of plasma benazeprilat concentrations after oral administration of benazepril

Parameter	Control group 1st day	Control group 7th day	Ascites group 1st day	Ascites group 7th day
Peak concentration (*n*g/m*l*)	7 27.8 ± 21.2	7 33.0 ± 13.8	7 83.5 ± 75.9	7 140.8 ± 142.6
Time to peak concentration (hours)	7 3.3 ± 4	7 3.6 ± 2.4	7 5.7 ± 8.2	7 2.7 ± 1.3
$t_{1/2}$ (hours)	6 5.72 ± 6.16	6 4.95 ± 5.32	5 3.44 ± 5.05	5 6.74 ± 9.52
$AUC_{(0-24hr)}$ (ngh/m*l*)	7 176 ± 111	7 217 ± 50	7 548 ± 442	7 971* ± 839
Total body clearance (m//hr/kg)	7 3,489 ± 2,634	7 1,891 ± 593	7 1,305 ± 898	7 791* ± 558

Data are expressed as the number of dogs and mean ± standard deviation, *: significantly different from the value in the control group on the same experimental day, AUC: area under the concentration-time curves from 0 to 24 hr.

Table 4. Plasma angiotensin-converting enzyme activities before and after oral administration of benazepril

Variable	Group	1st day Time after administration (hours) 0	2	4	8	7th day Time after administration (hours) 0	2	4	8
Plasma ACE activity (IU/m*l*)	Control (n=7)	3.9 ± 1.6	1.6* ± 2.2	1.8** ± 1.8	2.0** ± 1.5	3 ± 1	0.9** ± 1.2	0.9** ± 1.4	2.7 ± 0.8
	Ascites (n=7)	4.0 ± 2.0	1.4** ± 1.4	1.6* ± 2.4	2.0 ± 2.7	4 ± 2	1.4** ± 1.3	0.6** ± 1.2	1.4** ± 2.2

Data are expressed as mean ± standard deviation, P: Probability of significant difference between the values on the 1st and 7th days, * and **: significantly different from the value before administration, $P<0.05$ and $P<0.01$, respectively.

ations 1 week after administration in dogs of the control and ascites groups. Furthermore, rectal temperature, heart rate, and respiration rate did not alter significantly in dogs of either group.

Laboratory test results: Table 5 shows laboratory test results on the 1st and 7th administration days. In dogs of the control group, there were no significant differences in any variable between the 1st and 7th administration days. Dogs of the ascites group had a slightly higher ALT and urea nitrogen concentration, and lower total protein and albumin concentrations. On the 7th day, the RBC and WBC counts, plasma activities of ALT and ALP, and plasma concentrations of urea nitrogen, creatinine, total protein, albumin, combined β- and γ-globulins, sodium, potassium, and chloride did not change significantly in dogs of the ascites group.

DISCUSSION

Some dogs of the ascites group had higher concentrations of benazeprilat and benazepril. Earlier elevation and attainment of higher levels of plasma benazepril concentrations might reflect higher absorption ability of benazepril by the intestine, lower first-pass metabolism in the liver, and possi-

Table 5. Laboratory test results on the 1st and 7th day of benazepril administration

Variable	Control group				Ascites group			
	n	1st day	7th day	P	n	1st day	7th day	P
Red blood cell	7	827	799	NS	7	891	766	NS
($\times 10^4/\mu l$)		± 127	± 105			± 256	± 162	
White blood cell	7	143	187	NS	7	122	129	NS
($\times 10^2/\mu l$)		± 35	± 49			± 39	± 28	
Alaninetransaminase	7	41	34	NS	7	81	78	NS
(U/*l*)		± 18	± 5			± 71	± 55	
Alkalinphosphatase	7	101	90	NS	7	157	174	NS
(U/*l*)		± 45	± 38			± 100	± 161	
Urea nitrogen	7	12.6	12.9	NS	7	32.3*	35.5	NS
(mg/d*l*)		± 0.9	± 2.4			± 15.1	± 28.9	
Creatinine	7	0.8	0.8	NS	7	1.1	1.2	NS
(mg/d*l*)		± 0.1	± 0.2			± 0.3	± 0.5	
Total protein	7	5.4	5.7	NS	7	4.5*	4.8	NS
(g/d*l*)		± 0.5	± 0.7			± 0.8	± 0.9	
Albumin	7	2.8	2.6	NS	7	2.2**	2.4	NS
(g/d*l*)		± 0.2	± 0.3			± 0.3	± 0.3	
β+γ-globulin	7	1.6	1.7	NS	7	1.6	1.7	NS
(g/d*l*)		± 0.5	± 0.5			± 0.3	± 0.5	
A/G ratio	7	0.91	0.87	NS	7	0.79	0.79	NS
		± 0.18	± 0.31			± 0.15	± 0.10	
Sodium	7	147	146	NS	7	145	144	NS
(meq/*l*)		± 3	± 2			± 4	± 5	
Potassium	7	4.0	4.0	NS	7	3.9	4.0	NS
(meq/*l*)		± 0.4	± 0.3			± 0.5	± 0.4	
Chloride	7	109	111	NS	7	108	106*	NS
(meq/*l*)		± 4	± 3			± 3	± 5	

Data are expressed as No. of dogs and mean± standard deviation, P: probability of significant difference from the value on the 1st administration day, NS: not significant, ** and *: significantly different from the value of the control group, $P<0.01$ and $P<0.05$.
β+γ-globulin: combined concentration of β- and γ-globulins.

ble port-caval shunting, as is suggested in human patients with compensated liver cirrhosis [1]. Normal or higher plasma benazeprilat concentrations suggest that the biotransformation ability from benazepril to benazeprilat is normal or even higher in dogs with ascitic heartworm disease in spite of liver injury. The absorption of benazepril from the intestine and its conversion to benazeprilat by the liver might not be peculiar in dogs with ascitic heartworm disease. In one dog of the ascites group, however, the plasma benazeprilat concentration increased 12 hr after administration. This result may be accidental, or it suggests that there are occasions when benazepril is absorbed slowly by the intestine. On the 7th day, however, plasma benazeprilat concentration in the same dog began to increase after 0.5 hr, and reached the maximum concentration after 4 hr, then decreased as in the other dogs.

Dogs with ascitic heartworm disease have impaired liver and kidneys [17, 19]. Benazeprilat is eliminated by urinary and biliary excretions [18]. The total body clearance tended to be lower in the ascites group, and plasma benazeprilat concentrations at 24 hr after administration were similar in both groups, but abnormal signs owing to benazepril administration could not be observed during the examination in either control or ascites dogs. Laboratory tests showed no abnormal alterations in dogs of the control or ascites group. It has been reported that benazepril has an excellent safety record in dogs with renal failure [9, 15]. Dogs with kidney dysfunction administered 20 fold of the given dose of benazepril [8] had higher plasma benazeprilat concentrations than dogs with ascitic heartworm disease, and they had no clinical problems. From the results of the present study, benazepril is useful for the suppression of plasma ACE activity in dogs with ascitic heartworm disease.

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