New Insights on Effect of Kidney Insufficiency on Disposition of Angiotensin-Converting Enzyme Inhibitors: Case of Enalapril and Benazepril in Dogs

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ABSTRACT

The influence of a renal injury on the disposition of benazeprilat, the active moiety of benazepril, and of enalaprilat, the active moiety of enalapril, two angiotensin-converting enzyme (ACE) inhibitors (ACEIs), having different routes of elimination in dog was investigated during a mild renal insufficiency obtained by a nephrectomy-electrocoagulation method reducing glomerular filtration rate by ~50%. Plasma concentrations of the active moieties were analyzed with a physiologically based model taking into account the binding to ACE (high affinity, low capacity). An influence of renal insufficiency on enalapril disposition was shown with an increase in its plasma concentration, which was correlated to the reduction of the glomerular filtration rate. No such effect was evidenced for benazeprilat. With the physiologically based model analysis, it was shown that renal impairment led to an increase of the apparent benazeprilat clearance (260%), whereas that of enalaprilat was reduced to 40 to 55%. Renal insufficiency had no significant effect either on the apparent volume of distribution of each drug or on the binding parameters [i.e., maximal binding capacity (Bmax) and affinity (Kd)]. Enalaprilat and benazeprilat inhibitory action on ACE also was evaluated ex vivo. Similar patterns of inhibition were observed for both drugs. Renal injury had no significant influence on the overall effect of benazeprilat, whereas the inhibition effect of enalaprilat was significantly increased. It was concluded that renal insufficiency may have effects on the ACEI disposition but that the measurable active moiety plasma concentration is not the most appropriate endpoint to describe and interpret the consequence of a renal injury on ACEI.

In humans, most of the angiotensin-converting enzyme (ACE) inhibitors (ACEIs) are cleared by the kidney via glomerular filtration or a combination of glomerular filtration and tubular secretion. Renal impairment causes significant changes in ACEI disposition and increases the circulating levels of the active moiety. This has led to recommendations to reduce both the dosage and frequency of ACEI administration to avoid higher circulating drug levels than those required for appropriate ACE inhibition (Begg et al., 1989; Hoyer et al., 1993). However, some ACEIs (e.g., benazeprilat) have both renal and hepatic elimination and offer a potential advantage in terms of safety for the management of subjects with renal failure (Waldmeier and Schmid, 1989). The active metabolite of benazepril is still significantly eliminated in end-stage renal failure, suggesting that the nonrenal clearance can compensate for the absence of renal excretion (Kaiser et al., 1989). This contrasts with enalaprilat for which the levels detected at the steady state were largely increased compared with those in first dosing in patients with severely impaired renal function (Kelley et al., 1984; Kelly et al., 1985; Oguchi et al., 1993; Hersh et al., 1996).

In fact, the need or not for a dosage regimen adjustment of ACEI during kidney insufficiency should take into account not only the route of drug elimination but also the actual meaning of the measured plasma drug concentration both in terms of ACE inhibition and of nonspecific ACEI effects (other than ACE inhibition). The meaning of the plasma ACEI concentration is difficult to express due to the rather complicated disposition of ACEI. In humans, it is generally accepted that the plasma concentration time profiles for ACEIs such as enalaprilat and perindoprilat have two main phases: an initial elimination phase that reflects renal (or nonrenal) clearance of the free fraction of the active moiety (e.g., enalaprilat) and a protracted phase that reflects release of the active moiety from saturable binding to tissue and plasma ACE (Francis et al., 1987; Lees et al., 1989; MacFadyen et al., 1993). Thus, the terminal phase is not a classical elimination phase and, theoretically, is not involved in a possible bioac-
cumulation of ACEI, which is governed by the initial elimination phase.

The relationship between the initial phase of ACEI disposition and the drug elimination has been experimentally confirmed in humans for spiraprilat, the increase in area under the plasma concentration curve (AUC) with renal impairment being associated with a prolongation of the initial elimination phase rather than the terminal phase of the plasma concentration time profile (Meredith et al., 1994). This general aspect of ACEI disposition also has been demonstrated for benazeprilat in dogs, the elimination half-life of the free fraction being ~30 to 40 min, whereas the slope of the terminal phase, which represents the benazeprilat binding to ACE, is ~10 to 14 h (Toutain et al., 2000).

Another aspect of ACEI disposition that has not been adequately documented in renal impairment is the relationship between the actual overexposure of the drug (as evaluated from the measured plasma concentrations) and the ACE inhibition. Current analytical techniques measure the plasma-free drug (i.e., the fraction that is actually free and non-specifically bound to plasma albumin) and the fraction of the drug that is specifically bound to the binding sites located in the plasma (i.e., ACE) (see below). Because this binding is saturable, there is no simple parallelism between the measurable plasma drug concentration and that of the target ACE (plasma or tissular) that is bound by the ACEI. This means that a higher AUC of the plasma-measurable drug is not necessarily associated with a proportionally higher or longer drug effect.

Finally, renal impairment can modify other relevant pharmacodynamic parameters such as the concentration of converting enzyme, its affinity to the ACEI, and its plasma versus tissue location. This can affect not only the pharmacological response but also drug disposition because ACE-binding affinity affects elimination and, hence, the duration of ACEI action.

The present experiment was designed to compare the influence of an experimental renal injury on the disposition of benazeprilat, the active moiety of benazepril, that in dog has two routes of elimination (Waldmeier and Schmid, 1989) and enalaprilat, the active moiety of enalapril, that is mainly eliminated by the kidney (Tocco et al., 1982). The relationship between drug (free- or fraction-measurable drug by the analytical technique) and ACE inhibition was explored both in control and renal-impaired conditions with a pharmacokinetic/pharmacodynamic approach with the framework developed in Toutain et al. (2000). Part of the results were presented succinctly elsewhere (Lefebvre et al., 1999).

**Materials and Methods**

**Animals**

Ten female beagle dogs, from 8 months to 4.5 years old, weighing 10.5 to 12.5 kg at the start of the experiment were used. The dogs were fed once daily with a commercial dog food. Tap water was given ad libitum and the daily consumption was recorded.

**Experimental Design**

The dogs were randomly assigned, according to body weight, to two groups of five animals. The dogs were subjected to an initial two-period crossover experiment under control conditions separated by a washout period of 10 days. During period 1, benazepril or enalapril was administered to a given set of five dogs, the treatment being changed for the second period 10 days later. Two weeks after completion of the first crossover, mild renal failure was experimentally induced in all dogs. Seven to 11 days after surgery, the dogs were subjected to a second two-period crossover experiment to assess drug disposition under conditions of renal insufficiency. The order of drug administration was identical with that of the first crossover.

**Kidney Function Assessment**

Kidney function was assessed by measuring glomerular filtration rate (GFR) with iotalamate plasma clearance as marker. Iotalamate clearances were measured 1 week before each crossover test drug administration. Plasma biochemical variables (sodium, potassium, proteins, urea, and creatinine) were obtained three times under control conditions and three times after the experimental renal injury.

**Test Substances and Products**

Benazepril hydrochloride (mol. wt. 461), a prodrug of benazeprilat (mol. wt. 396.4) was supplied as 5 mg film-coated tablets (Fortekor 5; Novartis Animal Health, Basel, Switzerland). Enalapril (mol. wt. 493), a prodrug of enalaprilat (mol. wt. 348) was supplied as 5-mg tablets (Cardivet, Intervet, Cambridge, UK). Iotalamate meglumine (Contrix 28 Perfusion; Laboratoire Guerbet, Roissy-Charles de Gaulle, France) was supplied as a 600-mg/ml solution.

**Drug Administration and Blood Sampling**

The enalapril or benazepril dose given to all dogs was ~0 mg/kg (1437 nmol/kg for enalapril and 1261 nmol/kg for benazepril), i.e., one entire tablet of each drug was given by oral route. The exact doses (mean ± S.D.) of ACEI administered were 0.46 ± 0.02 and 0.45 ± 0.02 mg/kg enalaprilat and benazeprilaz, respectively, before surgery and 0.48 ± 0.03 and 0.48 ± 0.02 mg/kg after surgery. Blood samples were obtained before (0) and 1, 5, 10, 30, 60, and 90 min and 2, 3, 4, 6, 8, 10, 12, 24, 32, 48, and 72 h after ACEI administration. Blood was sampled from the jugular vein, placed in heparinized tubes, and centrifuged (1000g, 10 min) at 4°C. Aliquots of plasma were stored at −20°C until analysis. Iotalamate meglumine was administered by i.v. route at 80 mg/kg via an indwelling catheter placed in the cephalic vein. Blood samples were obtained before (0) and 2, 5, 10, 20, 30, 60, and 90 min and 2, 3, 5, and 6 h after iotalamate administration. Blood was sampled from the jugular vein, placed in a heparinized tube, and centrifuged (1000g, 10 min). Plasma was stored at −20°C until analysis. Blood samples for biochemical parameters were placed in tubes containing heparin lithium (Venoject; Terumo, Leuven, Belgium), and centrifuged (1000g, 10 min, 4°C). Plasma aliquots were stored at −20°C until analysis.

**Analytical Techniques**

Iotalamate concentrations were measured with the modified HPLC method described by Jayewardene et al. (1994). Specificity from endogenous compounds was verified on different blank plasma from control dogs. The linearity was demonstrated over a concentration interval of 5 to 1000 µg/ml. The within-day precision expressed by the relative standard deviation was <7%. The between-day precision was <15%. The accuracy range was between 87 and 112%. The limit of quantification was fixed at 5 µg/ml, the within-day precision was 6.35% and the accuracy was 89% for this value. The biochemical plasma parameters were determined with an analyzer (Ektachem 700 × R; Kodak, Johnson & Johnson Clinical Diagnostic Europe, Illkirch Graffenstaden, France).

The assays of enalapril and enalaprilat were performed by a commercial laboratory (Cepheac Research Center, St. Benoit, France) with combined liquid chromatography (LC)/mass spectrometry (MS) operating in positive chemical ionization mode. Enalapril, enalaprilat, and their internal standards (3H1)benazepril and 3H1benazeprilat) were extracted from plasma by solid-phase extraction on a C18 car-
tride. The residue was derivatized with diazomethane and then injected into the LC/MS/MS device. To quantitate the analytes present in the plasma extracts, the mass spectrometer was operated in multiplexed reaction monitoring. The eluent from the LC column was introduced into the mass spectrometer via the heated nebulizer interface generating the positively charged ions (m/z 391 for methylated enalapril and m/z 377 for methylated enalaprilat). MS/MS experiments were carried out with argon as the collision gas. The mass spectrometer was operated in such a way that the protonated parent ions (m/z 391 and m/z 377) were selected by the first quadrupole filter to be fragmented to daughter ions in the second quadrupole. The resulting fragment ions (m/z 317 for enalapril and enalaprilat) were analyzed with the third quadrupole.

The linearity of the method was verified over the 1 to 200 ng/ml range with a limit of quantification validated at 1 ng/ml with 0.5 ml of dog plasma. The intraday precision and accuracy of the LC/MS/MS procedure were determined at four concentration levels (1, 10, 20, and 200 ng/ml). The coefficient of variation ranged from 1.75 to 18.71% and the mean percentage of difference ranged from −6.85% to +11.29% for the two compounds. The interday precision and accuracy were verified after analysis of plasma samples spiked at the same four concentration levels in three batches. The coefficient of variation and the mean percentage of difference were <14% for both compounds.

The benazepril and benazeprilat assays were performed by Novartis Animal Health Inc. (Saint Aubin, Switzerland). Benazepril and benazeprilat were measured in plasma with a gas chromatography-MS method adapted from that described by Sioufi et al. (1988). The between-day coefficient of variation for precision was <10% and the limit of quantification was set at 1 ng/ml.

The ACE activity was determined in all the plasma samples obtained after benazepril and enalapril administrations with a commercial kit according to the manufacturer’s instructions (ACE Radiosassay System; Ventrex Labs, Portland, ME). This involved hippuryl-glycyl-glycine as substrate, one unit of activity corresponding to the amount of hippuric acid generated per minute per 1 ml of plasma. The coefficients of variation within and between days were 4.4 and 9.7%, respectively.

**Experimental Renal Injury**

Experimental renal failure was induced with a method adapted from Duffee et al. (1990) and described elsewhere (Lefebvre et al., 1999). Briefly, renal failure was induced by using a nephrectomy-electrocoagulation procedure. The right kidney was excised and portions of the left renal cortex were electrocoagulated by repeated 1-cm stabs with a stainless steel probe connected to an electrocautery unit. The number of stabs per kidney was 120 to induce moderate renal failure (i.e., 25–50% of normal glomerular filtration rate). All the animals recovered rapidly from surgery and regained normal activity within 24 h.

**Clinical Observation and Necropsy**

The animals were observed daily for general health according to a standardized clinical examination performed by a trained veterinarian. Dogs were weighed regularly. After completion of the second crossover, the animals were euthanized by i.v. administration of pentobarbital sodium. The remaining kidney was removed and placed in 10% formol until histopathological examination.

**Data Analysis**

Iotalamate plasma clearance (Cl) was obtained with a noncompartmental approach (eq. 1):

\[
Cl = \text{Dose} / \text{AUC}(0-\text{tlast})
\]

(1)

with AUC, the area under the plasma iotalamate concentration curve obtained by trapezoidal rule without extrapolation to infinity.

The decrease (D%) in glomerular filtration rate was determined by eq. 2:

\[
D\% = \frac{Cl - Cl_{\text{iotalamate}}}{Cl_{\text{iotalamate}}} \times 100
\]

(2)

with \(Cl\) and \(Cl_{\text{iotalamate}}\), the plasma iotalamate clearance before and after surgery, respectively.

For benazepril and enalapril and their active moiety, the AUC without extrapolation to infinity was calculated by arithmetic trapezoidal rule (Gibaldi and Perrier, 1982). The observed maximal concentration and its corresponding time (i.e., \(T_{\text{max}}\)) were directly obtained from the raw data. For enalapril and benazepril, the plasma concentrations were low and no other kinetic analysis was performed.

A conventional compartmental modeling approach leads to a systematic misfit (overprediction) of the first plasma concentration. In addition, the estimated lag time was consistently longer than the first time at which pharmacodynamic ex vivo activity was observed (data not shown). Thus, only the results obtained with the framework developed in Toutain et al. (2000), with a physiologically based model, are presented. Briefly, a monocompartmental model can be constructed assuming that unbound drug is the sole form eliminated with a rate constant \(k_1\) (h⁻¹) from a central compartment with a volume \(Vc\) (liters per kilogram). Enalaprilat and benazepril bound specifically to ACE (high affinity, low capacity) and nonspecifically to albumin (low affinity, high capacity). ACE is an ectopeptidase that appears in a soluble form in blood (circular form) but that is mainly bound to the plasma membrane of vascular endothelium (the so-called tissular form). Thus, the plasma enalaprilat and benazeprilat concentrations (hereafter termed measurable enalaprilat and benazeprilat) measured by analytical techniques correspond to the sum of free enalaprilat or benazeprilat, the enalaprilat or benazeprilat specifically bound to the circulating ACE, and the enalaprilat or benazeprilat bound nonspecifically to albumin.

The ACE (circular and tissue) is immediately accessible to enalaprilat or benazeprilat and for modeling purposes, all the ACE is located in the central sampling compartment. However, the enalaprilat or benazeprilat bound to tissue ACE (i.e., at the luminal surface of vessels) is not measured by the analytical technique. Because the soluble form of ACE is assumed to originate from the membrane-bound forms by proteolytic action (Hooper, 1993), it was assumed that the circulating and noncircularizing forms of ACE share the same binding parameters \(B_{\text{max}}\) (in nanomoles), the maximal binding capacity and \(K_S\) (in nanomoles), the dissociation constant, i.e., the free plasma enalaprilat or benazeprilat amount corresponding to half-saturation of the entire ACE pool. The circulating fraction (fcirc) of ACE, from 0 to 1, was estimated as a parameter of the model given the binding capacity between circulating ACE (i.e., fcirc × \(B_{\text{max}}\)) and tissue ACE (i.e., \(1 - \text{fcirc} \times B_{\text{max}}\)). \(B_{\text{max}}\) and \(K_S\) were estimated in terms of amount (nanomoles) but expressed in terms of concentration (nanomoles per liter) by dividing the estimated amount by the volume of distribution (liters per kilogram) of the free fraction, i.e., Vc (Toutain et al., 2000). In our model, it must be noticed that the free enalaprilat or benazeprilat corresponds to the truly free active moiety and the fraction that was nonspecifically bound to albumin.

A fifth-order Runge-Kutta method with variable step size was used to solve the model numerically. The parameters were obtained with REVOL, a derivative free Monte Carlo minimizing algorithm (Koepple and Hamann, 1980). The goodness of fit of the described model was assessed with least-square criteria. The data points were weighted by the inverse of the squared observed value (\(1/\text{E}^2\)). The apparent plasma clearance (Cl/F) (milliliters per kilogram per minute) of free enalaprilat or benazeprilat was calculated by

\[
\text{Cl/F} = \frac{B_{\text{max}} \times Vc}{F}
\]

(3)
with $K_{10}$ and $V_c$ as defined above and directly estimated by modeling and F, the unknown bioavailability.

The half-life of free enalaprilat and benazeprilat was calculated as

$$\text{Half-life} = 0.693/K_{10}$$  \hspace{1cm} (4)

with $K_{10}$ as defined above.

Concentration Effect Modeling. The individual data obtained after oral benazepril and enalapril administration were analyzed. Because a total inhibition of ACE was observed, the relationship between plasma benazeprilat concentrations (free or measured) and the ex vivo ACE activity (in arbitrary units) was described by the fractional $E_\text{max}$ model according to the equation

$$E(t) = E_0 \left(1 - \frac{C^n}{IC_{50}^n + C^n}\right)$$  \hspace{1cm} (5)

where $E(t)$ is the measured plasma ACE activity at time $t$, $E_0$ is the control ACE activity value, and $C$, is the free plasma benazeprilat concentration. The free plasma concentrations were obtained by solving the physiologically based model with individually fitted parameters up to the last observed effect, i.e., 72 h after the enalapril or benazepril administration. $IC_{50}^n$ is the free plasma enalaprilat or benazeprilat concentration required to produce 50% of the total inhibition and $n$ is a power term representing the steepness of the concentration effect curve. Individual data were fitted with a nonlinear least-square regression and the same algorithm as for kinetic data; uniform weighting factor was used.

Statistical Analysis. Statistical analyses were performed with STATGRAPHICS (5STSC, Rockville, MD). The values are reported as means ± S.D. or as median and range. Statistical analyses of the different parameters obtained after the oral administration of test drugs were carried out with a general linear model or one of its submodels (eq. 6).

$$Y_{ijklm} = \mu + Drug_i + Status_j + Sequence_k + Sequence(\text{dog})_{kl}$$
$$+ \text{Status(period)}_{jm} + Drug \times Status + Sequence \times Status$$
$$\times Status + \epsilon_{ijklm}$$  \hspace{1cm} (6)

With $Y_{ijklm}$, a value obtained with the $i$th drug for the $j$th status, during the $k$th sequence, on the $l$th dog during the $m$th period. $\mu$ is the overall mean, $Drug_i$ is the fixed effect of the $i$th drug (i.e., 1 or 2, i.e., enalapril or benazepril), and $Status_j$ is the fixed effect of the $j$th status (i.e., 1 or 2, i.e., before or after surgery). $Sequence_k$, the sequence or group effect is the fixed effect of the $k$th sequence (i.e., 1 or 2). $Sequence(\text{dog})_{kl}$ is the random effect of the $l$th dog during the $k$th sequence with $l = 1$ to 10 and $k = 1$ or 2. $\text{Status(period)}_{jm}$, the fixed effect of the $m$th period for the $j$th status with $m$ and $j = 1$ or 2. $Drug \times Status$ and $Sequence \times Status$ are interacting terms and $\epsilon_{ijklm}$ is the random error in observing $Y_{ijklm}$.

This model was used to detect a possible differential carryover effect between two consecutive periods by inspecting the sequence effect, which is totally confounded with a differential carryover effect. The statistical test used to test this hypothesis of a sequence effect, was an F test equal to the ratio of the mean square of the sequence effect over the mean square of the random sequence (dog) effect. There was no evidence of a differential carryover effect, validating the crossover design for any of the pharmacokinetic or pharmacodynamic variables. There was no significant period effect. A submodel of the aforementioned general model was used for the subsequent data analysis. The drug effect was tested separately for each status with drug, period, sequence, and dog nested in sequence as factors. The status effect (before and after renal injury) was studied separately for each drug with a submodel that included status and drug as factors. The equality of variance of the groups to determine ANOVA applicability was tested with the Bartlett test. In the case of unequal variance ($P < .05$), a nonparametric ANOVA appropriate for matched samples (Friedman two-way ANOVA by ranks) was performed with drug or status as main factor.

In a second step, the data were analyzed to detect the relationship between drug disposition and renal status (as measured by GFR or creatinine plasma concentrations) and prodrug exposure (i.e., enalapril and benazepril AUC). This was done by regression with a linear (i.e., $y = ax + b$) or a power (i.e., $y = ax^n$) function, by multiple regression, partial correlation analysis, and covariance analysis. It must be remembered that a partial correlation coefficient measures the relationship between two variables while controlling the possible effects of other variables (by removing any linear relationships with other variables before calculating the correlation coefficient between the two variables of interest). The drug effect at different time was determined with ANOVA for repeated measurements. $P < .05$ was considered significant and .05 < $P < .1$ also were reported as indicating the possible effect (see Discussion). The results were concluded to be nonsignificant when $P > .1$.

Results

Effects of Surgery and Renal Failure on Biological and Clinical Parameters

Some transient clinical signs were observed but disappeared within 5 days of surgery. No modification of appetite (meal refusal) was observed during the study, except in the few days after surgery. A statistically significant ($P < .001$), but biologically irrelevant decrease in body weight of the dogs was observed after surgery (10.4 ± 0.5 versus 11.0 ± 0.5 kg). A significant increase of mean daily water consumption was observed after surgery (444 ± 167 versus 268 ± 126 ml) ($P < .001$) with a sharp increase observed during the first week after surgery (627 ± 178 ml/day).

No significant variation ($P > .05$) after surgery was observed for sodium, potassium, and plasma protein concentrations. A highly significant ($P < .001$) increase in urea (from 4.4 ± 0.8 to 10.4 ± 2.7 mM) and creatinine (from 78.5 ± 9.3 to 146.3 ± 32.1 μM) plasma concentrations was observed after surgery.

A significant decrease ($P < .001$) in glomerular filtration rate (determined by the inolamate plasma clearance) was induced by surgery (3.3 ± 0.7 ml/kg/min under the control conditions versus 1.7 ± 0.3 ml/kg/min in the renal-impaired dog). The decrease in glomerular filtration rate ranged from 32 to 59%, with a mean value of 48%.

Pathological examination of the remaining kidney revealed that the lesions were quantitatively and qualitatively similar in all dogs. They generally consisted of a locally induced infarction.

The linear correlation coefficient between urea and creatinine plasma concentrations was very high ($r = 0.974$, $P < .001$) and creatinine was selected as primary index of the actual renal insufficiency. The relationship between GFR and plasma creatinine was not linear but curvilinear. A multiplicative model provided an appropriate fit with a correlation coefficient of $r = -0.93$ ($P < .001$).

Effect of Renal Failure on ACEI Disposition

Statistical analysis did not reveal any sequence or period effect for either crossover (i.e., before and after surgery), for any of the investigated parameters.

Enalapril and Benazepril. After a single oral drug administration, concentrations increased rapidly, $T_{\text{max}}$ being observed within ~45 and 30 min for enalapril and benaze-
The plasma concentrations of enalapril and benazepril were below the level of quantification (~6 and 2 h after the drug administration regardless of the renal status of the animals. The AUC for both enalapril and benazepril was increased but not significantly for benazepril (P > .1). For enalapril, P was .067, indicating a possible effect in the renal-impaired dog; mean ± S.D. values are given in Table 1 for AUC, T\text{max}, and C\text{max} of the two prodrugs.

There was no significant correlation between the benazepril AUC and GFR or creatinine plasma concentration (r = 0.16, P > .1). In contrast, the enalapril AUC was significantly correlated to the GFR (r = −0.43, P = .057) (multiplicative model) and plasma creatinine concentration (r = 0.52, P = .019) supporting the conclusion that renal insufficiency had an effect on enalapril disposition (Fig. 2).

**Enalaprilat and Benazeprilat: Descriptive Analysis.**

Mean values of the plasma benazeprilat and enalaprilat concentrations versus time before and after renal injury are shown in Fig. 3. Both active moieties could begin to be quantified in plasma on average 30 to 45 min after drug administration. In control conditions, the measured C\text{max} values were 43.9 ± 32.9 and 55.0 ± 26.4 ng/ml for enalaprilat and benazeprilat, respectively (P > .1).

A significant variation in AUC but not in C\text{max} was observed for enalaprilat in the renal-impaired dog. The AUC for the measurable plasma enalaprilat concentrations increased from 23 589 ± 14 722 to 42 436 ± 20 853 ng·min/ml (P < .01). All but one dog showed an increase from 34 to 405% over control value, with a mean increase value of 149% (n = 9). It is noteworthy that the AUCs displayed large intersubject variations. Mean C\text{max} was increased in the renal-impaired dog (43.9 ± 32.9 versus 59.1 ± 23.3 ng/ml), but this variation was not statistically significant (P > .1). Renal failure had no significant effect on AUC for measurable plasma benazeprilat concentrations (13 790 ± 9 829 versus 14 879 ± 4 977 ng·min/mL, P > .1), but C\text{max} decreased significantly after surgery (from 55.0 ± 26.4 to 31.9 ± 17.7 ng/ml, P < .05) (Table 2).

**Enalaprilat and Benazeprilat: Physiologically Based Model Analysis.** Benazeprilat and enalaprilat plasma concentrations were analyzed with the physiologically based model, before and after renal injury. Figure 4 shows the observed plasma concentrations and the fitted curve for enalaprilat and benazeprilat before and after surgery for a rep-

![Fig. 1. Mean value of plasma concentration of enalapril and benazepril in 10 dogs before (●) and after (○) a surgically induced renal failure, after a single oral administration of enalapril or benazepril at a nominal dose of 0.5 mg/kg.](image)

![Fig. 2. Relationship between GFR or plasma creatinine concentrations and enalapril or benazepril area under the plasma concentration versus time curve profile.](image)

![Table 1. Pharmacokinetic parameters for benazepril and enalapril after a single oral administration of enalapril or benazepril at a nominal dose of 0.5 mg/kg before and after an experimental renal injury in 10 dogs (noncompartmental analysis) (mean ± S.D.).](table)
renal injury (no significant difference between the two drugs after the
(drugs but the difference was only significant for benazeprilat.
\(P\) between the two drugs (whereas that of enalaprilat was reduced to 40 to 45\% (\(P\)
ance: that of benazeprilat was increased by 260\% (\(5
plasma concentrations was greater for most of the dogs,
physiologically based model. After surgery, the scatter of

time) before and after renal injury are given

Fig. 3. Mean plasma concentration of enalaprilat and benazeprilat in 10
dogs before (●) and after (○) a surgically induced renal failure, after a
single oral administration of enalapril or benazepril at a nominal dose of
0.5 mg/kg.

representative dog. Visual inspection of the curves indicates that
the data were well fitted with the equation corresponding to
a physiologically based model. After surgery, the scatter of
plasma concentrations was greater for most of the dogs,
which often made fitting more difficult. The parameters
derived from this model before and after renal injury are given in
Table 3.

Renal impairment led to opposite effects on apparent clear-
ance: that of benazeprilat was increased by 260\% (\(P = .019\)),
whereas that of enalaprilat was reduced to 40 to 45\% (\(P = .058\)). Renal impairment had no significant effect on the
apparent volume of distribution of either drug (\(P > .1\)).

The large increase in the plasma clearance of benazeprilat
explained the reduction of the elimination half-life from 34 ±
19 to 18 ± 9 min (\(P = .096\)). The reduction of the apparent
plasma clearance of enalaprilat was associated with an
equivalent reduction of the apparent volume of distribution,
thus explaining that the plasma elimination half-life was not
modified by renal impairment (61 ± 48 versus 70 ± 33 min)
(\(P > .1\)).

Under control conditions, \(B_{\text{max}}\) did not differ significantly
between the two drugs (\(P > .1\)) with an overall mean value of
150 ± 62 nM. After the renal injury, \(B_{\text{max}}\) increased for both
drugs but the difference was only significant for benazeprilat
(\(P = .007\)) (Table 3). As under control conditions, there was
no significant difference between the two drugs after the
renal injury (\(P > .1\)).

Under control conditions, most of the binding capacity was
located in tissue, the circulating fraction being similar for
enalaprilat (7.1 ± 4.3\%) and benazeprilat (9.3 ± 5.6\%) (\(P >
.1\)). The renal injury had no influence on this parameter for
enalaprilat, but for benazeprilat there was an increase in the
circulating fraction. However, estimation of this parameter
raised some difficulties for several data sets and should be
considered with caution.

Under control conditions, \(K_d\) (the plasma concentration of
free drug at 50\% binding saturation and a measure of drug
affinity for ACE) was higher for enalaprilat (7.39 ± 5.37 nM
or 21.2 ± 15.4 ng/ml) than for benazeprilat (4.21 ± 2.56 nM
or 10.6 ± 6.5 ng/ml) but the difference was not significant
(\(P > .1\)). The renal injury had no significant effect on this
parameter (\(P > .1\)) but in these conditions, the difference
between the two drugs became significant (\(P = .041\)) (Table
3).

The apparent half-life of drug invasion under control con-
ditions (prodrug absorption and bioconversion of the prodrug
into its active moiety) was significantly shorter for benazepri-
lat (33 ± 26 min) than for enalaprilat (151 ± 57 min) (\(P <
.01\)). The renal injury had no effect on this kinetic parameter
(\(P > .1\)).

The lag time was longer for benazeprilat than for enala-
prilat, but the difference was not significant (\(P > .1\)). The
renal injury did not modify this parameter (\(P > .1\)), but the
difference between the two drugs was significant under this
condition (\(P = .052\)).

Enalaprilat and Benazeprilat Kinetics Versus Renal Status
and Prodrug Exposure (Covariance, Regression, and
Correlation Analysis)

The influence of renal status on disposition of the active
drug moiety may be direct (i.e., by interfering with a physi-
ological process controlling enalaprilat or benazeprilat dispo-
sition) or indirect (i.e., acting throughout prodrug disposi-
tion). To elucidate any genuine relationship between
enalaprilat and benazeprilat disposition and renal status (as
measured by GFR or creatinine plasma concentration) and
prodrug exposure (i.e., enalapril and benazepril AUC), the
kinetic parameters were analyzed with covariance analysis,
simple linear correlation, and partial correlation analysis.

Covariance analysis with the prodrug AUC as a covariable
showed that renal injury had no direct significant effect on
enalaprilat Cl/F (1.63 versus 1.10 l/kg/h for control and renal
impaired conditions, respectively), whereas the benazeprilat
Cl/F was significantly increased from 3.33 to 10.08 l/kg/h
(\(P = .015\)).

The direct influence of renal insufficiency (as measured by
creatinine plasma concentration) on the actual drug disposi-
tion was only evidenced for the benazeprilat Cl/F, which was
positively and significantly correlated to plasma creatinine
concentrations (\(r = 0.53, P = .020\)) or negatively correlated to
GFR (\(r = -0.57, P = .029\)) (partial correlation analysis) (Fig.
5). Neither \(B_{\text{max}}\) nor \(K_d\) were correlated to plasma GFR or
plasma creatinine, and it was concluded from the covariance
analysis with prodrug AUC as a covariable that renal injury
had no influence on the binding parameters for either ena-
laprilat or benazeprilat (\(P > .1\)). GFR, creatinine, and the
prodrug exposure had no significant effect on the half-time of
absorption (bioconversion), and there was no evidence of an
effect of renal injury on these parameters (correlation anal-
ysis, covariance analysis, \(P > .1\)).

The measured plasma enalaprilat or benazeprilat AUC
(the so-called total AUC) is the parameter most often re-
ported to describe the influence of renal injury on ACEI
disposition. Covariance analysis with prodrug AUC as co-
variable showed that the renal injury had a significant effect
TABLE 2
Pharmacokinetic parameters of enalaprilat and benazeprilat after a single oral administration of enalapril or benazepril at a nominal dose of 0.5 mg/kg before and after an experimental renal injury in 10 dogs (noncompartmental analysis) (mean ± S.D.)
The data were analyzed by parametric or nonparametric ANOVA. P values were calculated to test the renal status effect (control versus renal failure) and the drug effect (enalaprilat versus benazeprilat).

<table>
<thead>
<tr>
<th>Parameters (Units)</th>
<th>Control</th>
<th>Renal Failure</th>
<th>P Value for Renal Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(0–tlast)</td>
<td>Enalaprilat</td>
<td>23.589 ± 14.722</td>
<td>42.496 ± 20.853</td>
</tr>
<tr>
<td>(ng/min/ml)</td>
<td>Benazeprilat</td>
<td>13.790 ± 9.829</td>
<td>14.879 ± 4.977</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>Enalaprilat</td>
<td>0.049</td>
<td>0.83</td>
</tr>
<tr>
<td>P value (drug effect)</td>
<td>Enalaprilat</td>
<td>43.9 ± 32.9</td>
<td>59.1 ± 23.3</td>
</tr>
<tr>
<td></td>
<td>Benazeprilat</td>
<td>55.0 ± 26.4</td>
<td>31.9 ± 17.7</td>
</tr>
</tbody>
</table>

* AUC, area under the measurable plasma concentration versus time curve calculated by the trapezoidal rule from time 0 to the last observed value.
* Cmax, observed maximal plasma concentration.

![Graphs of Enalapril and Benazepril](image)

**Enalapril and Benazepril Inhibitory Action on ACE**

Figure 6 illustrates the mean time course of ACE inhibition after administration of benazepril and enalapril before and after renal injury. The mean baseline plasma ACE activity before prodrug administration was similar for both drugs and there was no significant effect of renal injury (P > .1).

The overall inhibition of enalapril and benazepril was measured by the area under the effect versus time curve (the lower the value, the higher the overall effect). Renal injury had no significant influence on the overall effect of benazepril. In contrast, the inhibitory effect of enalaprilat was significantly increased by the renal injury over the first 24 h (2656 versus 1615 activity unit × h, P = .074). The influence of renal status persisted beyond 24 h but became statistically nonsignificant.

The absence of influence of renal injury on the overall effect of benazeprilat was confirmed by the absence of significant correlation between creatinine plasma concentration and the different AUC effects calculated from 0 to 72 h. In contrast, a significant negative correlation between creatinine plasma concentration and the overall effect persisted for enalaprilat from 0 to 24 h (r = −0.42, P = .067), indicating that the overall inhibition of the ACE increased with severity of the renal injury. The partial correlation coefficient analysis showed that the correlation with creatinine vanished when the prodrug AUC was taken into account. Figure 7 shows, for a representative dog, the fitted and observed effect of enalaprilat and benazeprilat free concentration before and after renal injury.

The IC50 of enalaprilat and benazeprilat, based on the predicted free plasma enalaprilat and benazeprilat concentrations (nanomoles per liter) versus time (hours) before (●) and after (○) a surgically induced renal failure, after a single oral administration of enalapril or benazepril at a nominal dose of 0.5 mg/kg in a representative dog. Data were analyzed with the physiologically based model.

**Discussion**

There are many reports that describe the influence of renal failure in humans on ACEI disposition and include enalapril (Kelley et al. 1984, 1985; Oguchi et al., 1993) and benazepril (Kaiser et al., 1989; Sioui et al., 1992). Despite the fact that most of the authors acknowledged that ACEI disposition was not classical (MacFadyen et al. 1993), they consistently used a conventional framework to analyze their data. For the first
The data were analyzed by parametric or nonparametric [i.e., Friedman (F)] ANOVA. P values were calculated to test the renal status effect (control versus renal failure) and the drug effect (enalaprilat versus benazeprilat). $B_{\text{max}}$, being a species, not a drug property, the influence of renal status also was tested by pooling the data obtained with both enalaprilat and benazeprilat. In these conditions, the influence of the renal status was significant ($P < .05$).

<table>
<thead>
<tr>
<th>Parameters (Units)</th>
<th>Control (mean ± S.D.)</th>
<th>Renal Failure (mean ± S.D.)</th>
<th>P Value for Renal Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Vc/F$ (l/kg)$^a$</td>
<td>Enalaprilat 1.94 ± 1.30</td>
<td>Benazeprilat 2.32 ± 0.92</td>
<td>.11</td>
</tr>
<tr>
<td>$Cl/F$ (l/kg/h)$^b$</td>
<td>Enalaprilat 1.91 ± 1.89</td>
<td>Benazeprilat 3.72 ± 2.48</td>
<td>.019</td>
</tr>
<tr>
<td>$t_{\text{lag}}$ elimination (min)$^c$</td>
<td>Enalaprilat 61 ± 48</td>
<td>Benazeprilat 34 ± 19</td>
<td>.003</td>
</tr>
<tr>
<td>$B_{\text{max}}$ (nmol/l)$^d$</td>
<td>Enalaprilat 161 ± 73</td>
<td>Benazeprilat 139 ± 51</td>
<td>.0073</td>
</tr>
<tr>
<td>$f_{\text{circ}}$ (%)$^e$</td>
<td>Enalaprilat 7.1 ± 4.3</td>
<td>Benazeprilat 9.3 ± 5.4</td>
<td>.082</td>
</tr>
<tr>
<td>$K_s$ (nmol/l)$^f$</td>
<td>Enalaprilat 7.39 ± 5.37</td>
<td>Benazeprilat 4.21 ± 2.56</td>
<td>.33</td>
</tr>
<tr>
<td>$t_{\text{lag}}$ absorption/bioconversion (min)$^g$</td>
<td>Enalaprilat 151 ± 57</td>
<td>Benazeprilat 33 ± 26</td>
<td>.003</td>
</tr>
<tr>
<td>Lag time (min)$^h$</td>
<td>Enalaprilat 11.0 [0–45]</td>
<td>Benazeprilat 19.4 [6.3–52]</td>
<td>.78</td>
</tr>
</tbody>
</table>

$^a$ $Vc/F$, apparent volume of distribution of the free fraction.

$^b$ $Cl/F$, apparent plasma clearance of the free fraction.

$^c$ $t_{\text{lag}}$, elimination, half-time of elimination of the free fraction.

$^d$ $B_{\text{max}}$, total binding capacity (circulating and noncirculating converting enzyme).

$^e$ $f_{\text{circ}}$, fraction (percentage) of total binding site ($B_{\text{max}}$) that is located in plasma.

$^f$ $K_s$, concentration of free drug producing saturation of 50% of converting enzyme (circulating and noncirculating).

$^g$ $t_{\text{lag}}$, absorption, half-life of absorption of prodrug and/or transformation of prodrug into drug.

$^h$ Lag time, lag time to absorption and/or transformation of prodrug into drug.

In the conventional approach, AUC is the pivotal index used to assess the influence of renal failure on ACEI disposition and an alteration of AUC cannot systematically be attributed to a model of apparent clearance. This is illustrated in the present experiment with an absence of variation in AUC for benazeprilat, whereas there was an actual 2.6-fold increase in its apparent clearance. The lack of repercussion of renal failure on the benazeprilat AUC was due to the concomitant increase in specific binding of the benazeprilat to ACE.

In experiments reporting the influence of renal failure on ACEI disposition in humans, the variation of the observed $C_{\text{max}}$ is generally reported to be correlated to $Cl\text{cr}$. In the perspective of the physiologically based model, the $C_{\text{max}}$ parameter provides information about the disposition of the free drug fraction. For high concentrations (with respect to $B_{\text{max}}$), ACE is saturated and above $B_{\text{max}}$, the drug kinetics become linear. In contrast to the total AUC, the value of $C_{\text{max}}$ is roughly dose-proportional and an alteration in Cl could be reflected by a proportional variation in $C_{\text{max}}$. In the present experiment this was obvious for benazeprilat with the AUC not modified, but the observed $C_{\text{max}}$ being significantly decreased due to an increase in the apparent Cl.

After an oral administration, the initial decay has been considered as the “true clearance phase” of the free fraction (MacFadyen et al., 1993), a view supported by the fact that renal impairment is often associated with a prolongation of the initial decay phase rather than the terminal phase of the plasma concentration profile (Meredith et al., 1994). The present experiment suggests another possible interpretation of the initial decay because there was evidence of a flip-flop situation (data not shown). The prolongation of the initial decay during renal impairment, which has been reported for several ACEI and in our experiment, could be due to alteration of the process of prodrug absorption and/or bioconversion rather than to a reduction of drug clearance.
Conventional modeling does not address the problem of the effect of renal failure on specific ACEI binding to ACE. In the present experiment, we have shown that the maximal specific-binding capacity ($B_{\text{max}}$) for both enalaprilat and benazeprilat was increased during renal failure ($\approx 26\%$). $B_{\text{max}}$ is not a drug property but a subject characteristic and its increase has to be taken into account whatever the ACEI selected. Our physiological model predicts that for any ACEI (with or without dual excretion) the measurable AUC should be increased in renal failure (versus normal renal function) provided that the other factors contributing to the AUC are unaltered. The physiological model also predicts that the influence of the $B_{\text{max}}$ increase should be more evident when the plasma concentrations are low (e.g., drug tested at a rather low dosage regimen or with a slow rate constant of absorption/bioconversion) because the influence of nonlinearity is greater at low plasma concentrations.

In contrast to $B_{\text{max}}, K_d$ is a drug property and expresses the drug’s affinity for ACE. In the present experiment, $K_d$ remained unaltered by the renal insufficiency. This suggests that the increase in $IC_{50}$ that was observed during renal insufficiency was not due to decreased drug binding to ACE but to some event at the stimuli response level.

In the present experiment, renal failure induced a significant increase of both the enalapril and enalaprilat AUC. In addition, the enalaprilat AUC was strongly correlated to the enalapril AUC, suggesting that enalaprilat was less eliminated by the renal route and more available for a biotransformation into enalaprilat by the liver. In contrast, benazepril is either directly eliminated by the liver or transformed (also by the liver) into benazeprilat (Waldmeier and Schmid, 1989), hence the renal failure has less impact on the disposition of this prodrug.

The measurable enalaprilat AUC was increased 2-fold, whereas the benazeprilat AUC remained unchanged, suggesting an absence of effect of renal failure on benazeprilat. The enalaprilat AUC increase can be explained by a reduction of the apparent enalaprilat clearance ($\approx 120\%$); an increase of the prodrug exposure, i.e., enalapril AUC ($\approx 30\%$); and an increase of the specific binding capacity ($\approx 27\%$).

The AUC of benazeprilat was not modified despite a clear-cut increase in apparent clearance that was positively correlated to creatinine. The origin of the clearance increase remains unclear.

The change in ACEI disposition in renal-impaired subjects can modify the relationship between drug dose and effect not only throughout drug exposure but also through a change in drug affinity and/or potency. In the present experiment, an ex vivo ACE inhibition assay was selected as the pharmacodynamic endpoint. The renal failure had no effect on the overall benazeprilat activity as judged by the area under the effect-time curve calculated from the time of administration to different times postadministration. In contrast, the enalaprilat overall effect was significantly increased, the influence of the renal failure being more evident when the first 24 h postadministration were considered. The increase of the enalaprilat overall effect is consistent with the increased overall enalaprilat exposure and the effects of both drugs were very similar under conditions of renal insufficiency. The same results were obtained in humans (Oguchi et al. 1993).

Using a pharmacokinetic/pharmacodynamic approach, the free plasma concentrations were directly and adequately cor-
In conclusion, the present experiment demonstrates that renal insufficiency may have many direct and indirect effects on the ACEI disposition, and the measurable active moiety AUC is probably not the most appropriate endpoint to describe and interpret the consequence of the renal injury. Instead, the use of a physiologically based modeling allows a more realistic description and interpretation of plasma concentration profiles, but due to the complexity of the underlying mechanism, dosage adjustment could be more easily done with pharmacodynamic endpoints rather than by interpreting the total measurable plasma drug concentrations.

Fig. 7. Observed (●) and fitted (−) enalaprilat and benazeprilat effect versus free drug concentrations obtained in a representative dog before and after renal injury. Visual inspection of the curves indicates that free drug plasma concentrations provide a suitable prediction of drug effect. For benazeprilat, renal injury largely decreased free plasma concentrations (note: the different scale for drug concentrations), whereas the converse was true for enalaprilat.

related to the ACE inhibition without requiring a hysteresis parameter. For benazeprilat, in control conditions, the mean IC_{50} (0.27 nM or 0.89 ng/ml) was nearly equal to that reported in Toutain et al. (2000) and was lower than the enalaprilat IC_{50} (1.09 nM or 3.1 ng/ml), confirming that benazeprilat is a more potent in vivo inhibitor than enalaprilat.

After renal impairment, the value of the IC_{50} was increased for both drugs but only significantly for enalaprilat. The origin of this increase was not an alteration of the drug affinity for ACE because the K_{a} remained unchanged. Whatever the origin of the IC_{50} increase, it damped the consequence of the overexposure associated with renal failure.

The altered pharmacokinetics of ACEI in chronic renal failure are considered a potential hazard and, in most instances, dosage adjustment is recommended, such as the necessity to reduce the dose to 50 or 25% of its usual value (for review, see Hoyer et al., 1993). However, due to the nonlinearity of ACEI disposition, the shape of the plasma concentration curve is important and a given total measurable AUC may correspond to very different situations in terms of the plasma concentrations versus effect relationship.