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Reabsorption and Metabolism of Quinapril and Quinaprilat in Rat Kidney: In Vivo Micropuncture Studies

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Abstract □ The tubular uptake and esterolysis of quinapril and quinaprilat were studied in male Sprague-Dawley rats using an in vivo micropuncture technique. [³H]Quinapril or [³H]quinaprilat was injected with [¹⁴C]inulin into either proximal or distal segments of the renal tubules, and urine was collected over 30 min. Urine and perfusate were assayed for [¹⁴C]inulin using dual label spectrometry. [³H]Quinapril and [³H]quinaprilat concentrations were determined in urine and perfusate using a reversed-phase HPLC procedure with radiochemical detection, coupled to liquid scintillation spectrometry. These studies demonstrated that quinapril could access the esterase enzyme from tubular fluid and be metabolized to quinaprilat in both proximal and to a lesser extent distal segments of the kidney tubule. Quinapril, but not quinaprilat, was extensively reabsorbed. Its reabsorption along the proximal tubule and/or the loop of Henle could account for as much as 45–50% of the available dose of quinapril. Further, the urinary recovery of quinapril and quinaprilat (after dosing quinapril into proximal segments) was urine flow rate dependent.

Introduction

Quinapril (Accupril, Parke-Davis) is a relatively new angiotensin converting enzyme (ACE) inhibitor that is used for the treatment of hypertension and congestive heart failure.^{1,2} Given its prodrug formulation, quinapril is enzymatically de-esterified in vivo to its more potent and pharmacologically active diacid form, quinaprilat.^{2,3} It is generally believed that the long-term response to ACE inhibitors is better reflected by inhibition of tissue ACE as opposed to plasma ACE activity.^{4,5} In this regard, the kidney may have an important role in modulating the pharmacologic response to quinapril and quinaprilat. For example, since all components of the

renin-angiotensin-aldosterone (RAA) system are also present within the kidney, desired or adverse effects on renal function may result from these ACE inhibitor-RAA interactions.⁶ Further, the systemic as well as local exposure to quinapril and quinaprilat will, in large part, be influenced by the renal disposition of drug and metabolite including its residence time and intrarenal metabolism within the tubular cell.

Previous studies in the isolated perfused rat kidney (rat IPK) have demonstrated that quinapril and quinaprilat are actively secreted into renal cells.^{7,8} In addition, less than 0.1% of quinapril is cleared as unchanged drug. Instead, over 99% of the drug is cleared as metabolite formed in the kidney. The rat IPK model is limited, however, in that it does not provide precise information on the disposition of drug species once it enters the tubular fluid. This knowledge is important because if tubular reabsorption is extensive, this process would effectively serve as a mechanism to maintain the body's reservoir of quinapril and therefore its pharmacologically active metabolite quinaprilat.

In an effort to further understand the complex renal disposition of ACE inhibitors, in vivo micropuncture studies were performed following the intratubular administration of quinapril and quinaprilat. In doing so, quantitative estimates of their tubular uptake and esterolysis could be obtained at distinct nephron segments.

Experimental Section

Preparation of Animals for Micropuncture—The kidney was prepared for tubule micropuncture using standard techniques.^{9–11} In brief, studies were performed in five male Sprague-Dawley rats (300–400 g) anesthetized with an intraperitoneal injection of thiobarbital (Inactin, 110 mg/kg body weight). Animals were infused intravenously with albumin-containing saline solutions to balance plasma losses associated with surgery. During the experiment, animals received a maintenance infusion of 4.5 mL/h (three animals) or 6 mL/h

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Table 1—Urinary Recovery of Quinapril and Quinaprilat: In Vivo Micropuncture Studies^a

Treatment	Location	N	Urine Flow ($\mu\text{L}/\text{min}$)	Recovery (%)		Total Recovery (%)	
				Quinapril	Quinaprilat		
Quinapril	Proximal	9	17.2 \pm 11.7	22.6 \pm 7.4 A	24.3 \pm 3.9 ABC	47.0 \pm 4.9 ABC	
Quinapril	Distal	5	12.6 \pm 6.8	78.3 \pm 2.7 A	10.8 \pm 2.2 ADE	89.1 \pm 3.8 A	
Quinaprilat	Proximal	6	11.4 \pm 9.6	—	91.8 \pm 1.4 BD	91.8 \pm 1.4 B	
Quinaprilat	Distal	4	13.8 \pm 12.5	—	97.0 \pm 6.8 CE	97.0 \pm 6.8 C	
Significance ^b				$p = 0.7283$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

^a Data are reported as mean \pm SD, where N represents the number of punctures in each treatment of three to five rats. [³H]Quinapril or [³H]quinaprilat were administered with [¹⁴C]inulin as bolus injections to proximal or distal segments of the rat tubule. Recovery data were calculated as $(U/P)_{\text{QH}}/(U/P)_{\text{14C}}$ in which U represents the cumulative amount of radiolabel recovered in urine (0–30 min) and P represents the injected amount of radiolabel in microinfusate. Quinaprilat data (after dosing quinapril) are reported in quinapril equivalent units. ^b p values were determined by ANOVA. For each parameter, significant differences among treatment groups are designated using common capital letters, as determined by Tukey's test.

(two animals) of isotonic saline. These rates were chosen to produce a mild diuresis. Body temperature was kept constant at 37.5 °C by placing the animals on a servo-controlled heating table. The left kidneys were exposed through a flank incision, placed in a Lucite cup for immobilization, and covered with warm mineral oil to prevent desiccation of the surface.

Study Design—Sites for proximal tubule microinjections were chosen at locations remote from peritubular capillary welling points where late proximal segments have been shown to converge. Passage of the stained injectate through several downstream segments confirmed that injection sites were always located in the early proximal to midproximal convolutions. Injection sites in the distal convoluted tubule were randomly selected. Microinjected volumes ranged from 20.8 to 81.6 nL and averaged 37.8 \pm 15.5 nL. Injections were made manually to avoid backflux of the injected fluid and were usually complete within 30 s. Beginning at the time of injection, urine was collected via a short ureteral catheter in 5–10-min intervals for a total time of 30 min. Urine from the contralateral kidney was also collected over the same time period as a control for completeness of the injection. Test solutions consisted of isotonic saline containing trace amounts (\approx 4 ng) of [³H]quinapril (specific activity 20.1 $\mu\text{Ci}/\mu\text{g}$, >98% radiochemical purity, gift of Parke-Davis) or [³H]quinaprilat (specific activity 22.2 $\mu\text{Ci}/\mu\text{g}$, >98% radiochemical purity, gift of Parke-Davis) and [¹⁴C]inulin (i.e., \approx 1000 ng; 2.5 $\mu\text{Ci}/\text{mg}$, ICN Biomedicals, Inc., Irvine, CA).

Analytical—Urine and perfusate samples were assayed for [¹⁴C]-inulin using dual label spectrometry. [³H]Quinapril and [³H]quinaprilat concentrations were determined in urine and perfusate using a specific and sensitive reversed-phase HPLC procedure with radiochemical detection, coupled to liquid scintillation spectrometry.¹² All samples had an activity of at least twice the background.

Data Analysis—The urinary recovery of labeled drug and metabolite, relative to inulin, was used to assess the extent of drug reabsorption and metabolism (i.e., via a mass balance approach). Specifically, recovery data were calculated as: $(U/P)_{\text{QH}}/(U/P)_{\text{14C}}$ in which U represents the cumulative amount of radiolabel recovered in urine (0–30 min) and P represents the injected amount of radiolabel in microinfusate.¹³ Data are reported as mean \pm SD unless otherwise indicated. For each parameter, statistical differences were determined between treatments by analysis of variance (ANOVA). Pairwise comparisons were then made using Tukey's test.

Results and Discussion

In vivo micropuncture experiments were performed in order to quantify the disposition of lumenally applied quinapril and quinaprilat along the rat tubule. A summary of the average results obtained in this study is given in Table 1. Following injection of quinaprilat into the proximal tubule, 91.8 \pm 1.4% of the administered dose was recovered in the urine in an unchanged form. The urinary recovery of quinaprilat was 97 \pm 6.8% when the substance was injected into the distal tubule.

Q : 100% of Dose

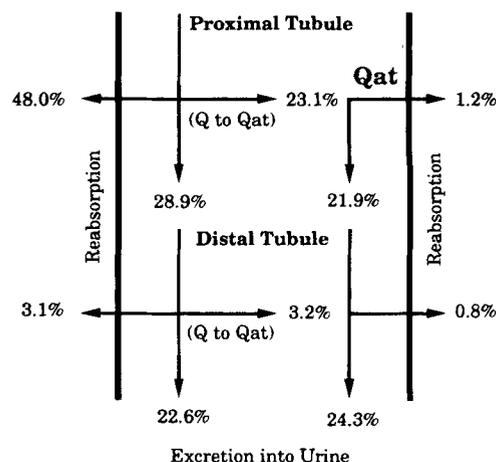


Figure 1—Micropuncture-based schematic for the urinary excretion, metabolism, and reabsorption of quinapril (Q) and quinaprilat (Qat) along the nephron tubule. Qat data are reported in Q equivalent units. Numerical values represent the percent of administered quinapril dose and were estimated using a mass balance approach, Table 1, and algebraic solutions. Thus, 22.6 and 24.3% were obtained from Table 1 directly; 28.9% was obtained from $22.6/0.783$; 3.1% was obtained from $(28.9 - 22.6\%) \times 0.4885$; 3.2% was obtained from $28.9 - 3.1 - 22.6\%$; 21.9% was obtained from $(x + 3.2\%) \times 0.97 = 24.3\%$; 0.8% was obtained from $21.9 + 3.2 - 24.3\%$; 23.1% was obtained from $(0.946x + 3.2\%) \times 0.97 = 24.3\%$; 48.0% was obtained from $100 - 23.1 - 28.9\%$; and 1.2% was obtained from $23.1 - 21.9\%$.

Thus, it could be estimated that about 5.4% of the dose of proximally delivered quinaprilat is reabsorbed along the proximal tubule and/or the loop of Henle while about 2.8% of the dose is reabsorbed along the distal convoluted tubule and/or the collecting duct.

After proximal administration of quinapril, only 22.6 \pm 7.4% of the injected dose was recovered in the urine as intact quinapril while 24.3 \pm 3.9% of the dose was recovered in urine as quinaprilat. Thus, about one-half the dose of proximally administered quinapril (i.e., 53.1%) was reabsorbed. In contrast, after administration of quinapril to distal tubular segments, 78.3 \pm 2.7% of the dose was recovered in the urine as unchanged drug and 10.8 \pm 2.2% was recovered in urine as the active metabolite quinaprilat. Reabsorption of quinapril along the distal nephron accounted for only 10.9% of the dose of distally delivered drug. A schematic representation of the micropuncture data following quinapril administration to proximal segments of the renal tubule is depicted in Figure 1. Aside from quinapril and quinaprilat, no other drug-related species were found in these studies.

Differences in the recovery of quinapril and quinaprilat, as a function of administration location or drug, were not due to differences in urine flow rate between the four treatment groups studied (Table 1; $p = 0.7283$). However, within a given treatment group, several significant relationships were observed. For example, when quinapril was administered to proximal segments, there was a significant positive correlation between quinapril recovery and urine flow rate (Figure 2A; $r = 0.878$, $p < 0.002$), a significant negative correlation between quinaprilat recovery and urine flow rate (Figure 2B; $r = 0.948$, $p < 0.001$), and a significant positive correlation between quinapril/quinaprilat recovery and urine flow rate (Figure 2C; $r = 0.938$, $p < 0.001$). On the other hand, when quinapril was administered to distal segments, statistically significant correlations were not observed between quinapril recovery and urine flow rate (Figure 3A; $r = 0.106$, $p > 0.50$), between quinaprilat recovery and urine flow rate (Figure 3B; $r = 0.384$, $p > 0.50$), and between quinapril/quinaprilat recovery and urine flow rate (Figure 3C; $r = 0.426$, $p > 0.20$). Likewise,

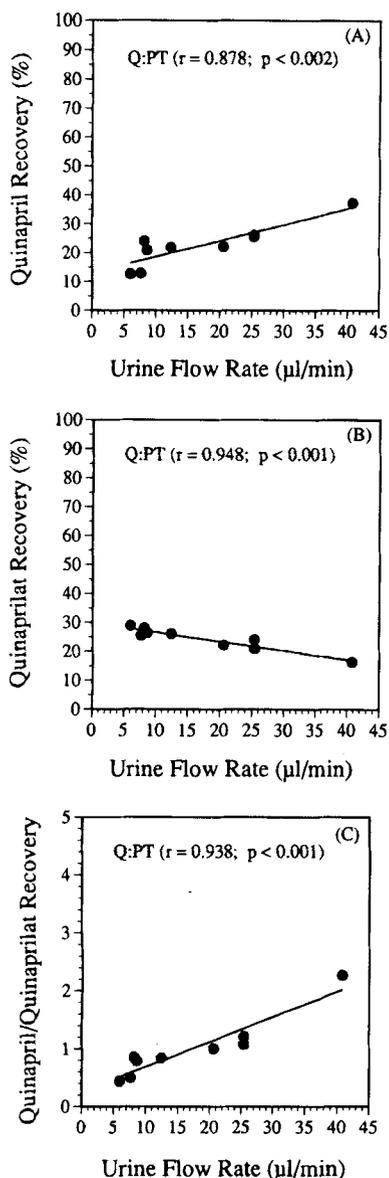


Figure 2—Recovery vs urine flow rate profiles of quinapril (A), quinaprilat (B), and quinapril/quinaprilat ratio (C) after administration of quinapril to proximal segments (Q:PT) of the renal tubule. Qat data are reported in Q equivalent units.

quinaprilat recovery was not significantly correlated with urine flow rate when quinaprilat was administered to either proximal (Figure 4A; $r = 0.108$, $p > 0.50$) or distal (Figure 4B; $r = 0.108$, $p > 0.50$) segments of the renal tubule.

Overall, two major observations have been made in this study. First, a significant fraction of intratubular quinapril can gain access to the esterase enzyme and be metabolized to quinaprilat, a process that occurs in both the proximal and to a lesser extent the distal tubule. And second, the renal handling of quinapril, but not of quinaprilat, includes an extensive component of tubular reabsorption. The reabsorptive movement of quinapril takes place primarily in proximal regions of the nephron (i.e., proximal convoluted and proximal straight tubules, thin descending and thick ascending limbs of the loop of Henle), and reabsorption in these regions can account for as much as 45–50% of the drug delivered to the proximal tubule.

This latter observation is of interest since one would expect passive reabsorption of the amphoteric drug quinapril, which is mostly anionic at physiologic pH, to be favored along the late nephron due to more favorable concentration gradients,

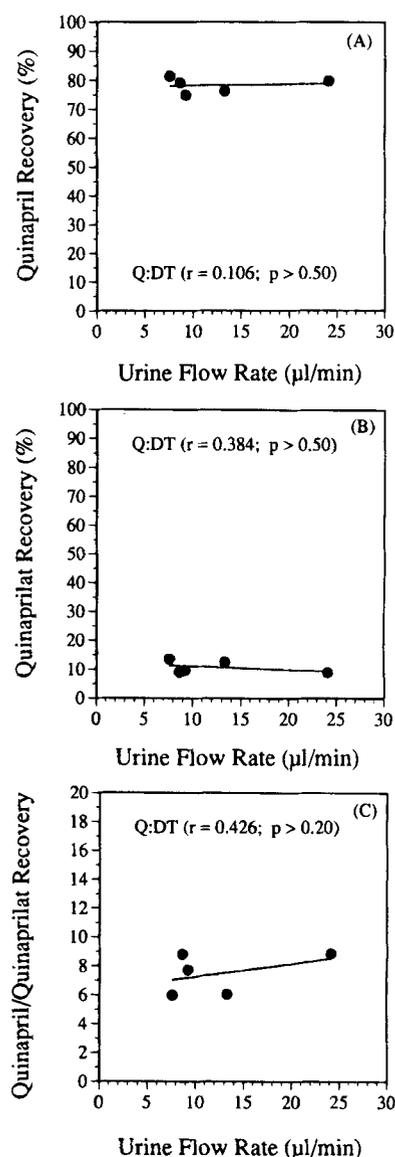


Figure 3—Recovery vs urine flow rate profiles of quinapril (A), quinaprilat (B), and quinapril/quinaprilat ratio (C) after administration of quinapril to distal segments (Q:DT) of the renal tubule. Qat data are reported in Q equivalent units.

residence times in the tubule, and acidic urine pH. Although speculative, it is possible that quinapril may be undergoing reabsorption by a carrier-mediated system (e.g., proton/peptide cotransport). This hypothesis is supported by the fact that quinapril is a Phe-Ala-Pro tripeptide analog and that other ACE inhibitors have been shown to utilize the di-tripeptide transporter in the intestine.^{14,15} It should also be appreciated that similar results were obtained in micropuncture studies with the dipeptide glycylsarcosine.¹³ In these studies, it was reported that 67% of the dipeptide was reabsorbed if microinfusion took place at the early or late section of the proximal convoluted tubule, but decreased to 18% if early distal tubule sections were microinfused. More definitive studies will be needed to address this issue, namely, the mechanism of quinapril's tubular reabsorption process.

Dependence of the renal disposition of quinapril on urine flow rate is consistent with at least two possible mechanisms. First, an increased urine flow rate may cause a reduced cellular uptake of quinapril, resulting in a reduction in the transtubular reabsorption of intact quinapril as well as a reduced delivery of the drug to an intracellular esterase enzyme. Alternatively, an increased urine flow rate may

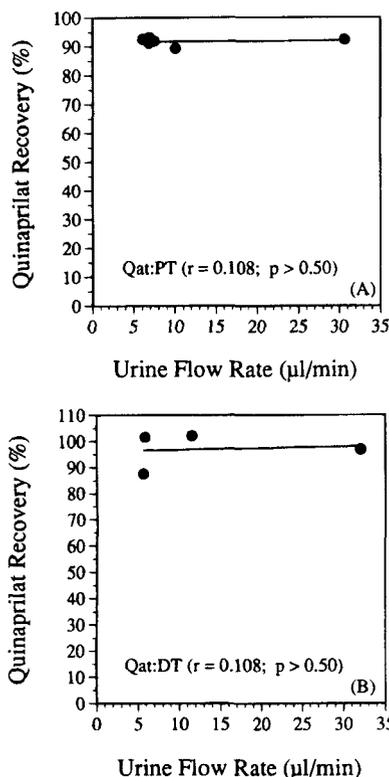


Figure 4—Recovery vs urine flow rate profiles of quinaprilat after administration of quinaprilat to proximal segments (Qat:PT) of the renal tubule (A) and after administration of quinaprilat to distal segment (Qat:DT) of the renal tubule (B).

reduce quinapril metabolism by an esterase bound to the luminal membrane. In both situations, an increased recovery of quinapril, a decreased recovery of quinaprilat, and an increased recovery of quinapril/quinaprilat would occur (as shown in Figure 2). Although this study was not designed to differentiate between these two mechanisms, the first scenario is more probable. This preference is based on the results of rat IPK studies^{7,8} in which quinapril was shown to be extensively transported into renal tubular cells, yet less than 0.1% was cleared as intact drug.

A similar pattern of renal handling was observed for enalapril in the isolated red blood cell-perfused rat kidney.^{16,17} Using single-pass IPK data with a physiological model, the influx and efflux clearances of enalapril at the basolateral membrane were greater than or equal to the plasma flow rate, yet only 6% was cleared as prodrug. Instead, 94% of enalapril was converted by the rat kidney to its pharmacologically active metabolite enalaprilat. In addition, the authors¹⁶ reported that the overall urinary clearance mechanism of enalapril was net reabsorption while enalaprilat was cleared via net filtration. However, intrarenal metabolism may mask one's ability to interpret urinary excretion data for enalapril and other drugs metabolized by the kidney,¹⁸ and as a result, additional experimental approaches are necessary. Given the more polar nature of enalaprilat as compared to quinaprilat and that quinaprilat is reabsorbed to a limited extent ($\leq 10\%$ in these studies), it is likely that the former ACE inhibitor should have

little or no reabsorption. This speculation is confirmed by in vivo rat studies in which the unbound clearance of enalaprilat was reduced to that of glomerular filtration alone in the presence of probenecid.¹⁹

In conclusion, it appears that in addition to quinapril's ability to be secreted into and metabolized by renal tubular cells, 40–50% of proximally delivered prodrug is reabsorbed in the proximal regions of the nephron. As a result of this extensive reabsorption mechanism, it is likely that the systemic and/or local residence times of quinapril and quinaprilat (via conversion by the liver, kidney, and other extravascular tissues) are increased, thus augmenting the efficacy of ACE inhibition. Although it is difficult to speculate on how these renal transport/metabolic processes effect modulation of the pharmacological response to drug, several important factors need to be considered. These would include, in part, the relative contributions of carrier-mediated transport in the secretory and reabsorptive directions, the identification and characterization of involved transporters, and the precise sites and extent of metabolic conversion by renal esterase.

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