Effects of Organic Anion, Organic Cation, and Dipeptide Transport Inhibitors on Cefdinir in the Isolated Perfused Rat Kidney

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Cefdinir (Omnicef; Abbott Laboratories) is a cephalosporin antibiotic primarily eliminated by the kidney. Nonlinear renal elimination of cefdinir has been previously reported. Cefdinir renal transport mechanisms were studied in the erythrocyte-free isolated perfused rat kidney. Studies were performed with drug-free perfusate and perfusate containing cefdinir alone to establish the baseline physiology and investigate cefdinir renal elimination characteristics. To investigate cefdinir renal transport mechanisms, inhibition studies were conducted by coperfusing cefdinir with inhibitors of the renal organic anion (probenecid), organic cation (tetraethylammonium), or dipeptide (glycylsarcosine) transport system. Cefdinir concentrations in biological samples were determined using reversed-phase high-performance liquid chromatography. Differences between treatments and controls were evaluated using analysis of variance and Dunnett’s test. The excretion ratio (ER; the renal clearance corrected for the fraction unbound and glomerular filtration rate) for cefdinir was 5.94, a value indicating net renal tubular secretion. Anionic, cationic, and dipeptide transport inhibitors all significantly affected the cefdinir ER. With probenecid, the ER was reduced to 0.59, clearly demonstrating a significant reabsorptive component to cefdinir renal disposition. This finding was confirmed by glycylsarcosine studies, in which the ER was elevated to 7.95, indicating that reabsorption was mediated, at least in part, by the dipeptide transporter system. The effects of the organic cation tetraethylammonium, in which the ER was elevated to 7.53, were likely secondary in nature. The anionic secretory pathway was found to be the predominant mechanism for cefdinir renal excretion.

Cefdinir (Omnicef; Abbott Laboratories) is an extended-spectrum third-generation cephalosporin approved for use in the United States, Japan, and several countries in Europe. Prescribed for use in treating mild to moderate bacterial infections in adults, children, and infants, cefdinir demonstrates excellent activity against a wide range of gram-positive and gram-negative bacteria. Cefdinir MICs have been reported to be comparable or superior to those of cefaclor and cefuroxime (9). Cefdinir elimination is primarily mediated by the kidney, and nonlinear clearance has been reported (20, 24). In the study by Richer et al. (24), renal clearance (CLR) decreased with increasing doses in healthy adults. In that study, the CLR values were 2.78, 2.14, 2.10, and 1.97 ml/min/kg for 200-, 300-, 400-, and 600-mg doses, respectively. This observation is consistent with previous reports that β-lactam drugs are eliminated by glomerular filtration, as well as anionic and cationic carrier-mediated secretory systems, in the kidney (5, 40). Furthermore, it has been shown in vitro that some dicarboxylic, α-amino, and non-α-amino β-lactams, including cefdinir, can be recognized by the carrier-mediated dipeptide transporters PEPT1 and PEPT2 (12, 36, 37).

Given the nature of cefdinir elimination from the body, the nonlinear cefdinir clearance previously reported may be attributable to one or more saturable renal elimination processes, including active tubular secretion and reabsorption. Moreover, the complex nature of cefdinir renal elimination could potentially affect the dosing regimen, safety, or efficacy profile of cefdinir or other coadministered drugs whose elimination is primarily mediated by the kidney. Concomitant drug administration may affect the systemic exposure, clearance, and circulatory half-life of cefdinir or the coadministered drug due to drug-drug competition for renal carrier-mediated transport. Indeed, Jacolot et al. (13) previously reported such an interaction between cefdinir and the angiotensin-converting enzyme inhibitors captopril and quinapril. In that study, the pharmacokinetic interaction was so strong between quinapril and cefdinir that no cefdinir elimination phase was detectable in four of six rats studied. The authors concluded that this observation was probably due to competition at the renal tubular anionic carrier level, resulting in a significantly decreased CLR for cefdinir. Results from the study demonstrate how the effi-
cacy and safety of cefdinir or coadministered drugs may be significantly altered by drug-drug interactions in the kidney, which play a major role in the homeostasis of endogenous substances and the elimination of xenobiotics from the body. The potential for drug-drug interactions in the kidney, combined with the effects of age, nutritional status, and disease state on the kidney’s ability to effectively eliminate xenobiotics, makes it important to understand the factors that influence the renal elimination of drugs such as cefdinir.

The present study was conducted to explore the factors influencing the renal disposition of cefdinir. The erythrocyte-free isolated perfused kidney (IPK) of the rat is a durable model for CLR in which renal transport and/or metabolism has been studied for a wide range of clinically relevant compounds. We used the IPK model, since it can be subjected to various physiologic, biochemical, or pharmacological alterations in a controlled manner and systemic influences on renal function can largely be eliminated. In addition, specific and precise data on CLR and factors altering renal clearance mechanisms can be readily generated (2, 15, 16, 19, 25, 26, 30). In this regard, the individual contributions of filtration, secretion, reabsorption, and metabolism to the CLR can be determined by the use of competitive and metabolic inhibitors. With respect to clinical relevance, the rat IPK has provided information that is mechanistically consistent with that observed in humans for many drugs, including amiloride (31), cimetidine (22, 31), aceazolamide (34), methotrexate (32), lamivudine (33), quinapril and quinaprilat (16), procainamide (22), and dyphylline (1).

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (325 to 447 g; Charles River Laboratories, Wilmington, Mass.) were used as kidney donors for all IPK experiments. The animals were housed and cared for under controlled conditions with the supervision of the University of Michigan’s Unit for Laboratory Animal Medicine. The animals were acclimated in housing at the Unit for Laboratory Animal Medicine for at least 3 days before experimentation. Food and water were provided ad libitum.

Surgical procedure for kidney harvest. The experimental approach was adapted from methods originally described by Nishitatsusuki-Uwo et al. (21) and Bowmer (3) and subsequently revised by Kugler et al. (15, 16). Briefly, the rats were anesthetized with sodium pentobarbital (65 mg/kg of body weight intraperitoneally), and after the induction of deep anesthesia, an intravenous bolus injection of heparin-mannitol (200 U and 180 mg) was administered. The abdominal vascular supply was then isolated, and the major abdominal blood vessels were cut. The perfusate composition due to the collection of urine and perfusate samples were continuously monitored not to damage the capsular surface of the kidney.

Initial perfusion pressure during the equilibration period was slightly higher but fell as hemodynamic equilibration was achieved. Following initiation of perfusion and harvest of the kidney, the organ was placed in the IPK apparatus and a 15-min period for homeostatic equilibration was allowed to pass. The experimental period began (t = 0) with the addition of 150 µl of [14C]inulin to the recirculating perfusion medium (16.7 µCi/ml; specific activity, 2.5 µCi/µg). In all IPK studies, cefdinir (5 µM) and potential transport inhibitors were dissolved separately in a small volume of perfusate and added to the recirculating medium immediately following the addition of [14C]inulin. A 15-min postdose equilibration period was then allowed for drug distribution and hemodynamic stability to occur. Following this period, the remaining 90 min of the experiment was divided into 10-min urine collection intervals for the evaluation of physiologic and biochemical parameters. Urine was collected into tubing that was connected with, a 1-ml tuberculin syringe. Perfusion (1.5 ml) was withdrawn from the sampling port with a 3-ml syringe (21-gauge needle) at the midpoint of each clearance interval (every 10 min). The perfusate and urine pHs were determined immediately after collection. During the experimental period, changes in perfusate composition due to the collection of urine and perfusate samples were determined by chromatographic analysis with modified Krebs-Henselet buffer and blank perfusion medium (no inulin or other compounds present, respectively. Data from the postdose equilibration period (t = 0 to 15 min) were not included in the mean calculations or statistical evaluations.

The parameters evaluated as descriptors of overall renal function included the urine flow rate, urine pH, perfusate flow rate, perfusate pH, perfusion pressure, renal vascular resistance (RVR), glomerular filtration rate (GFR), filtration fraction, and fractional excretion of glucose (FE glucose) and sodium (FE Na+). Cefdinir studies were performed in the absence of inhibitors to characterize the CLR of cefdinir alone in the IPK. Cefdinir inhibition studies were conducted in the presence of known competitive inhibitors of the renal organic anion (probenecid; PRO), organic cation (tetraethylammonium; TEA), and dipetide (glycylsarcosine [Gly-Sar]) transport systems. Samples of the perfusate and urine were analyzed for concentrations of cefdinir, inulin, glucose, and sodium, as described below.

Protein binding. Perfuse samples collected during the actual IPK experiments (cefdinir with and without inhibitors) were subjected to ultrafiltration. Protein-free ultrafiltrate was obtained from perfuse using a disposable microconcentrator (Centrifree, Amicon Division, W. R. Grace & Co., Danvers, Mass.) and centrifugation. The device employs an anisotropic hydrophilic YM7 membrane that excludes molecules larger than ~30 kDa. Briefly, a 475-µl aliquot
of perfusate was added to the device, which was then capped, equilibrated at 37°C for 15 min in a 35°C fixed-angle rotor, and then centrifuged for 25 min at 37°C and 1,800 × g. When necessary, the perfusate pH was adjusted prior to ultrafiltration to the original value obtained and recorded at the time of sample collection. Adjustment of the pH was made by gassing the sample with CO₂ or by removing CO₂ by vortexing the sample. Preliminary studies (data not shown) demonstrated that cefdinir was not appreciably bound to the ultrafiltration device and that protein leakage during the ultrafiltration process did not occur. Therefore, the fraction unbound of cefdinir (FU) in perfusate was calculated as the ratio of the cefdinir concentration in the ultrafiltrate to that in the perfusate.

**Analytical methods.** Cefdinir concentrations in IPK urine, perfusate, or ultrafiltrate samples were determined using specific and sensitive reversed-phase high-performance liquid chromatographic (HPLC) techniques. 8-Chlorotheophylline was used as the internal standard (ISTD). Samples were prepared by precipitating proteins using a 25% (wt/vol) solution of trichloroacetic acid in the mobile phase. Following centrifugation, the supernatant was decanted into amber autosampler vials with plastic inserts, loaded into an autoinjector maintained at 10°C, and analyzed by HPLC.

Cefdinir was separated from perfusate or ultrafiltrate sample components on a 3-µm C₁₈ column using a helium-sparged mixture of 50 mM citrate-phosphate buffer (pH 2.6) and acetonitrile as the mobile phase (91:9 [vol/vol]). The mobile phase was pumped isocratically at a rate of 1.2 ml/min. The column temperature was maintained at 50°C. The chromatographic eluent was monitored spectrophotometrically at a λ of 287 nm. Absorbance peak/height ratios (cefdinir-ISTD) for calibration standards were analyzed by regression analysis using a linear model with concentration as the independent variable. The regression equation, weighted using 1/(concentration)², did not include and was not forced through the origin. Sample cefdinir concentrations were calculated using the regression equation. The range of quantification was 0.025 to 10.0 µg/ml for a 0.125-ml IPK perfusate or ultrafiltrate sample and 0.250 to 50.0 µg/ml for a 0.025-ml IPK urine sample. Assay performance (validation) was assessed prior to sample analysis by examining the assay precision (percent relative standard deviation [%RSD]) and accuracy (percent relative error [%RE]) over three or four batch analyses. IPK perfusate, ultrafiltrate, and urine samples (calibration and quality controls) were within ±8.0% of their intended values (%RE), and the precision was ≤10.0% (%RSD).

Following HPLC assay validation, sample cefdinir concentration values were accepted only if at least four of six quality control (QC) samples were within ±15% of the respective mean cefdinir concentration value calculated for the QC during assay validation. Additionally, if two QCs at the same concentration level failed, the data were not reported.

Glucose concentrations (in milligrams per deciliter) in perfusate or urine were determined using a model 27 glucose analyzer (Yellow Springs Instruments, Yellow Springs, Ohio). The instrument was calibrated prior to use and zeroed as necessary between injections. The accuracy of replicate (n = 16) measurements of calibration standards, as assessed by mean %RE, was within ±0.9% of theoretical values. Precision, as assessed by %RSD, was ≤1.1%.

**FIG. 1.** Schematic illustration of the erythrocyte-free recirculating rat IPK apparatus. The chamber is maintained at 37°C. The arrows indicate the direction of perfusate flow.
TABLE 1. Physiologic-parameter values from control and cefdinir IPK experiments

<table>
<thead>
<tr>
<th>Expt\textsuperscript{a}</th>
<th>n</th>
<th>Rat wt (g)</th>
<th>Urine flow rate (ml/min)</th>
<th>Urine pH</th>
<th>Per fusate flow rate (ml/min)</th>
<th>Kidney perfusate pressure (mm Hg)</th>
<th>RVR (mm Hg min/ml)</th>
<th>Per fusate pH</th>
<th>GFR (ml/min)</th>
<th>Filtration fraction (%)</th>
<th>FE glucose (%)</th>
<th>FE Na\textsuperscript{+} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no drug)</td>
<td>9</td>
<td>377 (7.3)</td>
<td>0.081 (18.3)</td>
<td>4.63 (2.6)</td>
<td>47.1 (16.1)</td>
<td>81 (3.7)</td>
<td>1.77 (19.8)</td>
<td>7.39 (0.3)</td>
<td>0.761 (17.6)</td>
<td>1.62 (9.3)</td>
<td>2.60 (37.7)</td>
<td>9.0 (17.9)</td>
</tr>
<tr>
<td>CEF</td>
<td>9</td>
<td>391 (9.8)</td>
<td>0.108 (22.9)</td>
<td>6.48 (1.5)</td>
<td>46.1 (10.8)</td>
<td>80 (5.0)</td>
<td>1.76 (18.2)</td>
<td>7.39 (0.3)</td>
<td>0.877 (20.1)</td>
<td>1.92 (24.5)</td>
<td>3.05 (16.1)</td>
<td>10.7 (15.9)</td>
</tr>
<tr>
<td>CEF + PRO (500 \textmu M)</td>
<td>8</td>
<td>387 (9.1)</td>
<td>0.116 (17.0)</td>
<td>6.51 (1.2)</td>
<td>49.5 (9.7)</td>
<td>79 (6.8)</td>
<td>1.50 (16.0)</td>
<td>7.40 (0.3)</td>
<td>0.833 (17.3)</td>
<td>1.69 (17.2)</td>
<td>2.29 (20.1)</td>
<td>11.0 (30.0)</td>
</tr>
<tr>
<td>CEF + PRO (5,000 \textmu M)</td>
<td>8</td>
<td>395 (3.4)</td>
<td>0.118 (19.6)</td>
<td>6.55 (1.1)</td>
<td>49.2 (5.3)</td>
<td>78 (2.6)</td>
<td>1.59 (8.2)</td>
<td>7.39 (0.3)</td>
<td>0.772 (20.3)</td>
<td>1.58 (21.5)</td>
<td>3.81 (16.5)</td>
<td>13.5 (14.8)</td>
</tr>
<tr>
<td>CEF + TEA (500 \textmu M)</td>
<td>7</td>
<td>375 (5.8)</td>
<td>0.094 (14.1)</td>
<td>6.59 (2.3)</td>
<td>46.0 (9.6)</td>
<td>78 (2.6)</td>
<td>1.72 (9.3)</td>
<td>7.39 (0.3)</td>
<td>0.827 (22.2)</td>
<td>1.80 (21.1)</td>
<td>2.41 (24.1)</td>
<td>9.9 (12.3)</td>
</tr>
<tr>
<td>CEF + Gly-Sar (500 \textmu M)</td>
<td>6</td>
<td>387 (10.6)</td>
<td>0.122 (21.3)</td>
<td>6.64 (2.3)</td>
<td>46.8 (9.6)</td>
<td>78 (3.8)</td>
<td>1.68 (12.5)</td>
<td>7.40 (0.1)</td>
<td>0.941 (8.1)</td>
<td>2.02 (8.9)</td>
<td>2.74 (34.3)</td>
<td>11.1 (18.9)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} CEF, cefdinir.  
\textsuperscript{b} Statistically different from cefdinir alone (P < 0.05). Initial cefdinir perfusate concentrations were 5 \textmu M in all CEF experiments.

RESULTS

In all experiments, the quantity of cefdinir added to the perfusate was intended to achieve an initial drug concentration that was therapeutically relevant (5 \textmu M, or 2.0 \textmu g/ml). In low-dose (500 \textmu M) PRO experiments, the intended concentration of PRO was approximately equal to that achieved following therapeutic-dose (\textleq 700 \textmu M) administration. High-dose (5,000 \textmu M) PRO experiments were used to probe the presence and extent of renal tubular reabsorption. TEA was used as a mechanistic probe and has no known therapeutic use. Gly-Sar is a model dipeptide that is resistant to hydrolysis, and it was studied at the perfusate concentrations expected for endogenous dipeptides (17, 28, 29).

No major (>5% total height) interfering chromatographic peaks coeluted with cefdinir or the ISTD in any matrix of any experiment during the sample analysis. The concentrations of cefdinir in the perfusate declined steadily and in a log-linear fashion during the experimental period.

The mean physiologic and mean pharmacokinetic data from IPK experiments are summarized in Tables 1 and 2, respectively. Although the physiologic parameter values were generally consistent for all treatments, two parameter values were statistically different from those in the cefdinir control experiment. Kidney perfusate pressure for the low-dose PRO treatment was significantly lower than that of cefdinir controls. Nevertheless, the GFR (a measure of functional nephron mass) and FE glucose (a measure of proximal tubular transport) remained consistent for this and all other treatments. Additionally, since the other key indices of IPK function were

TABLE 2. Pharmacokinetic-parameter values from cefdinir IPK experiments

<table>
<thead>
<tr>
<th>Expt\textsuperscript{a}</th>
<th>n</th>
<th>FU (%)</th>
<th>FU \textsuperscript{-} GFR (ml/min)</th>
<th>CL\textsubscript{R} (ml/min)</th>
<th>CL\textsubscript{R}/GFR</th>
<th>CL\textsubscript{R}/FU (ml/min)</th>
<th>ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEF</td>
<td>9</td>
<td>3.1</td>
<td>32.2</td>
<td>0.027 (32.2)</td>
<td>0.145 (23.8)</td>
<td>0.168 (18.9)</td>
<td>5.08 (35.6)</td>
</tr>
<tr>
<td>CEF + PRO (500 \textmu M)</td>
<td>8</td>
<td>3.4</td>
<td>18.9</td>
<td>0.028 (17.6)</td>
<td>0.025 (16.0)</td>
<td>0.036 (4.6)</td>
<td>0.78 (26.6)</td>
</tr>
<tr>
<td>CEF + PRO (5,000 \textmu M)</td>
<td>8</td>
<td>13.0</td>
<td>16.9</td>
<td>0.100 (26.2)</td>
<td>0.059 (23.9)</td>
<td>0.075 (14.3)</td>
<td>0.46 (24.5)</td>
</tr>
<tr>
<td>CEF + TEA (500 \textmu M)</td>
<td>7</td>
<td>2.8</td>
<td>10.3</td>
<td>0.023 (25.0)</td>
<td>0.170 (35.0)</td>
<td>0.201 (16.8)</td>
<td>6.33 (32.5)</td>
</tr>
<tr>
<td>CEF + Gly-Sar (500 \textmu M)</td>
<td>6</td>
<td>2.7</td>
<td>8.1</td>
<td>0.025 (13.0)</td>
<td>0.197 (19.5)</td>
<td>0.209 (14.2)</td>
<td>7.43 (15.1)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} CEF, cefdinir.  
\textsuperscript{b} Statistically different from cefdinir alone (P < 0.05). Initial cefdinir perfusate concentrations were 5 \textmu M in all experiments.
that observed for cefdinir alone. Experiments with inhibitors were similar in pattern. However, the ER values of cefdinir in all IPK experiments with PRO and FE Na⁺ (due to increased Na⁺ excretion in the presence of inhibitors varied widely and were all significantly different from that of cefdinir alone, with the exception of TEA experiments. GFR- or FU-normalized CLR values exhibited a similar pattern. However, the ER values of cefdinir in all IPK experiments with inhibitors were significantly different from that observed for cefdinir alone.

**DISCUSSION**

With the exception of kidney perfusate pressure (500 μM PRO) and FE Na⁺ (5,000 μM PRO), physiologic-parameter values for the IPK control, cefdinir alone, and cefdinir-plus-inhibitor experiments were similar to each other, consistent with those reported in the literature (2, 15, 16, 19, 25, 26), and reasonably stable over the entire duration of each experiment. Increased Na⁺ excretion for the high dose of PRO may be related to the elevated concentrations of this organic anion in the renal tubular lumen and peritubular capillaries. High PRO concentrations may favor sodium excretion in two ways. First, elevated intracellular sodium concentrations may tend to reduce sodium uptake into the cell at the apical membrane by the sodium-proton antiporter. Cefdinir binding to bovine serum albumin in the rat IPK, while slightly higher in comparison, is consistent with serum protein binding results previously reported in vivo for the rat at −90% (27). The significantly higher FU of cefdinir observed in the 5,000 μM PRO experiments is probably a result of cefdinir displacement by the high dose of PRO. In experiments with cefdinir alone, high protein binding in conjunction with the observed GFR resulted in a filtration clearance (FU·GFR) that was low. The CLR of cefdinir was approximately fivefold higher than filtration clearance alone, indicating that the drug undergoes substantial secretion in the kidney. When CLR is normalized to both FU and GFR, net tubular secretion is clearly demonstrated, as evidenced by a mean excretion ratio of 5.94 for cefdinir.

As a result of coperfusion with 500 μM PRO, the mean cefdinir CLR decreased dramatically, falling 83% (P < 0.005). Mean GFR- or FU-normalized CLR values were similarly affected, with reductions of 82 and 85%, respectively. In addition, the mean ER was reduced to less than unity (0.93; P < 0.005), a value not only indicating that had cefdinir secretion been substantially reduced but, more importantly, suggesting that there may be a reabsorptive component to cefdinir renal disposition as well. Overall, these results are consistent with those of the cefdinir-only experiments in that the ER values clearly demonstrated a secretory component to renal disposition.

Given that cefdinir interacts with the dipeptide transporters PEPT1 and PEPT2 (8, 36) and that cefdinir ER values were below unity in the 500 μM PRO experiments, additional stud-
ies were conducted at higher PRO concentrations to further evaluate the potential for cefdinir reabsorption in the kidney. As a result of coperfusion with 5,000 μM PRO, the FU of cefdinir increased by greater than fourfold, to 13% (P < 0.005). Since the GFR remained nearly constant compared to that of cefdinir alone, the increase in unbound cefdinir resulted in a mean cefdinir filtration clearance value that was increased by 266% (P < 0.005). Moreover, the mean CLR decreased 60% from that of cefdinir alone (P < 0.005), despite the large increase in filtration clearance. Even when normalized for FU (CLR/FU), the CLR fell 91% relative to that of cefdinir alone (P < 0.005). Taking both FU and GFR into consideration, the mean ER of cefdinir was reduced even further than with the low dose of PRO, to 0.59. This value, well below unity, clearly demonstrates a considerable reabsorptive component to cefdinir renal disposition. In fact, assuming that secretion had been reduced to zero by high-dose PRO administration, the fraction of cefdinir minimally reabsorbed from the tubular lumen (Frecabs) can be estimated as follows: Frecabs = 1 − CLR FU × GFR = 1 − ER = 1 − 0.59 = 0.41 (i.e., at least 41%).

Taking the PRO results together, the present data demonstrate that cefdinir tubular secretion is substantial, that cefdinir tubular secretion is inhibitable by PRO, and that this secretion is probably mediated by the renal organic anion secretory pathway. Nevertheless, previous studies have shown that some cephalosporins (e.g., cephalexin) may undergo renal tubular secretion mediated by the renal organic cation transport system (11, 40). Moreover, it should be noted that previous reports have concluded that organic anion and organic cation carrier-mediated transport systems in the kidney do not sense the degree of ionization of the substrate (38, 39). Therefore, additional studies were performed to more fully understand the nature of cefdinir renal tubular secretion and to evaluate the potential for cefdinir to undergo secretion by way of the organic cation-carrier-mediated transport system.

Following coperfusion of cefdinir with the organic cation TEA, mean CLR values trended higher relative to those observed for experiments with cefdinir alone, although they failed to reach statistical significance. These results were not due to changes in protein binding or filtration clearance, since the mean cefdinir filtration clearance did not increase in the presence of TEA. The same trend was apparent for FU-normalized CLR. However, once the CLR of cefdinir was normalized to filtration clearance (i.e., ER), the results clearly demonstrated an increase in cefdinir renal elimination. Accordingly, the mean ER of cefdinir increased 27% in the TEA treatment group relative to that of cefdinir alone (P < 0.05).

Our results demonstrate that an organic cation can actually increase the renal excretion of an organic anion to a statistically significant extent. One hypothesis to explain this observation may be that TEA forms an ion pair with cefdinir in the tubular lumen, thereby forming a complex that is unavailable for reabsorption, increasing cefdinir elimination. Another possible explanation may be that the effect of TEA on cefdinir renal disposition is secondary to the effect of TEA on the renal cationic transport system (Fig. 2). In this hypothesis, basolateral entry of TEA increases the intracellular concentration of TEA, which subsequently causes the apical membrane TEA-proton antiporter system to cause an overshoot of H+ into the cell. Higher-than-normal intracellular H+ could create a proton concentration gradient less favorable to the reabsorption of cefdinir by way of the proton-dipeptide cotransport system. Alternatively, since TEA is a known inhibitor of ATPase-sensitive K+ channels (23), TEA could impair cefdinir renal reabsorption by indirectly influencing the sodium-proton antiporter and proton-coupled symport of the drug. However, it might then be expected to have an inhibitory effect on organic anion secretion, via the sodium-α-KG cotransporter and basolateral α-KG–organic anion exchanger, resulting in a reduced ER. Regardless of the mechanism at work, this interaction may be important in predicting and understanding the potential for drug-drug interactions between substrates whose physicochemical properties, particularly ionization state, are distinct.

The increased ER of cefdinir in the presence of TEA demonstrates that cefdinir does not undergo tubular secretion mediated by the renal organic cation transport system shared by TEA. The apparent lack of cefdinir transport by the renal organic cation transport pathway is not entirely unexpected, since cephalosporin anti-infectives known to be substrates for this carrier-mediated transport system (e.g., cephalexin) have zwitterionic or cationic functionality not present in cefdinir (11, 40). Our results are more consistent with those reported by Tamai et al. (35), who presented evidence from rat renal cortex brush border membrane vesicles that cefixime, a close structural analog to cefdinir, is not transported by the renal organic cation transport system. Moreover, the present results from PRO and TEA coperfusion experiments with cefdinir are consistent with the physicochemical properties of the drug. According to the Henderson-Hasselbach equation, cefdinir (Fig. 3) is an anionic substrate at physiologic pH (Fig. 4). In addition, previous studies of rats and humans suggest that the renal disposition of cefdinir is, at least partially, under the influence of carrier-mediated secretory transport (13, 24). In humans and rats, cefdinir CLR exceeds filtration clearance (FU · GFR), suggesting that cefdinir undergoes net tubular secretion in the kidney. In healthy adults, a normal GFR value is ~1.8 ml·min⁻¹·kg⁻¹ (6). In the study by Richer et al. (24), cefdinir CLR was dose dependent and decreased from 2.78 to 1.97 ml·min⁻¹·kg⁻¹ as the single oral dose of cefdinir increased from 200 to 600 mg/kg in healthy adult human subjects. Combining these observations with available data on human serum protein binding (FU, ~35%), net renal tubular secretion is strongly suggested. These results are also consistent with a report by Brown (5), in which the author reviewed studies involving the concomitant use of PRO with each of 19 cephalosporins (excluding cefdinir). The results overwhelmingly demonstrated that the pharmacokinetics of 15 of the 19 cephalosporins tested can be significantly affected by coadmini-
istration with PRO. Impairment of cephalosporin CL\textsubscript{R} with coadministration of PRO was the predominant finding, strongly suggesting inhibition of renal organic anion secretion. Of the four compounds in which an interaction was not observed, all had bulky side chains at the 3 position of the cephalosporin nucleus, unlike the smaller vinyl moiety present in the same position in cefdinir. Our results with IPK are consistent with these previous findings and, taken together with our TEA results, clearly show that cefdinir renal secretion is primarily mediated by the renal organic anion transport system. Furthermore, the results of the PRO experiments strongly suggested a reabsorptive component to cefdinir renal elimination.

Supporting the latter hypothesis, coperfusion of cefdinir with 500 \mu M Gly-Sar resulted in statistically significant changes in the renal disposition of cefdinir. Although cefdinir protein binding and GFR were not significantly different from those of cefdinir alone, the mean CL\textsubscript{R} increased 35\% ($P < 0.05$). The mean FU- or GFR-normalized CL\textsubscript{R} was similarly increased. Mean CL\textsubscript{R}/FU values increased 46\% ($P < 0.005$) and mean CL\textsubscript{R}/GFR values increased 24\% relative to those of the cefdinir control ($P < 0.05$). Mean ER values also increased significantly, rising 34\% ($P < 0.02$). The present results for Gly-Sar are consistent with the experimental results for the high-dose PRO treatments. Taken as a whole, our results not only support a significant reabsorptive component to cefdinir renal disposition but also indicate that the mechanism of reabsorption involves, at least in part, the renal dipeptide transporter system. In contrast, given the drug’s poor lipophilicity (logP $\approx$ 3.47; pH 7.4) (18) and extensive ionization at urine pH values observed in the IPK, it is unlikely that significant passive tubular reabsorption of cefdinir would occur.

The observation that cefdinir undergoes reabsorption by the dipeptide transporter system in the kidney is a new finding. This observation is supported by a previous study (8) demonstrating the interaction of anionic cephalosporins with the low-affinity intestinal (PEPT1 in Caco-2 cells) and high-affinity renal (PEPT2 in SKPT cells) proton-coupled oligopeptide co-transporters. A similar interaction was found between \beta-lactam antibiotics in LLC-PK1 cells stably transfected with PEPT1 or PEPT2 (36). In both studies (8, 36), cefdinir inhibited Gly-Sar uptake, but with low affinity, resulting in 50\% inhibitory concentrations (or $K_i$ values) on the order of 10 to 20 mM for both transporters. Given the anticipated concentrations of cefdinir in the proximal tubular fluid ($\approx$2.9 to 13.7 \mu M, based on urine concentrations in the IPK and water reabsorption considerations), PEPT2-mediated drug reabsorption would be favored over that of PEPT1. However, the low-affinity interactions reported for both transporters suggest that cefdinir reabsorption may be low. This apparent discrepancy may reflect differences in studying cefdinir transport directly in a whole IPK versus inhibition studies in cell lines and transfected cell culture systems.

In summary, cefdinir is predominantly secreted in the kidney, and this secretion is mediated by a renal organic anion transporter(s) shared by PRO. Cefdinir elimination (ER) by the kidney can be increased by coadministration with TEA, most likely through an indirect effect. Cefdinir is also significantly reabsorbed by the kidney, and this reabsorption is most likely mediated by a renal dipeptide transporter(s). The fraction of cefdinir reabsorbed in the renal tubule is estimated to be 41\% or greater, a finding previously unreported in the literature. These findings suggest that in the clinical situation, systemic exposure of cefdinir may be increased by coadministration with anionic drugs and/or metabolites or decreased by

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Fraction_un-ionized_pH.png}
\caption{Fraction un-ionized versus pH for each of three acid-base functional groups in cefdinir. The $pK_a$ values are 1.9 (carboxylic acid group), 3.3 (amino group), and 9.9 (hydroxyl group).}
\end{figure}
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