Influence of Angiotensin II-Induced Alterations in Renal Flow on Excretion of Cefonicid in Isolated Perfused Rat Kidneys

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The effects of variations in renal perfuse flow on the excretion of cefonicid was examined in isolated perfused rat kidneys. Cefonicid, an expanded-spectrum cephalosporin, is primarily eliminated by active tubular secretion and is neither metabolized nor reabsorbed in the isolated kidney. We used angiotensin II (AII), a strong vasoconstrictor hormone of the afferent and the efferent arterioles in the kidney, to determine whether the renal and secretion clearances, as well as the excretion ratio (ER = CLw/fu × GFR), where CLw is renal clearance, fu is the unbound fraction, and GFR is glomerular filtration rate), of this low-extraction compound can be altered by a decreased perfusion flow. Control studies were performed in the absence (n = 5) and presence (n = 4) of AII; cefonicid studies were performed in the absence (n = 4) and presence (n = 5) of AII. AII (1 to 4 ng/min) and cefonicid (5 to 10 μg/min) were infused into the perfusate. Cefonicid was assayed by high-performance liquid chromatography, and its protein binding was determined by ultrafiltration. All decreased the perfuse flow rate and increased the renal vascular resistance and filtration fraction of the isolated kidney in the presence and absence of cefonicid. The glomerular filtration rate remained unchanged among the groups. The fractional excretion of glucose was low and steady, indicating a well-preserved tubular function. Although the unbound fraction was unchanged between treatments, the renal and secretion clearances and the excretion ratio of cefonicid were reduced by about 40% in the presence of AII (excretion ratios, 10.3 without AII versus 6.03 with AII). These results suggest that the altered clearance parameters of cefonicid are the result of a flow-induced change in the intrinsic secretory transport of the kidney.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (weight, 350 to 450 g) were used as kidney donors for the isolated perfused rat kidney preparations. All animals were allowed free access to food and water before experimentation. They were anesthetized with sodium pentobarbital (50 μg/g of body weight by the intraperitoneal route) prior to surgery.

Surgical procedure. The surgical approach that was adopted for the isolated perfused rat kidney system is based on the methods of Nishiisutsui-Uwo et al. (18) and Bowman (5), with minor modifications (11).

Perfusate composition. The kidney was perfused with a medium containing 6% previously dialyzed fraction V bovine serum albumin (ICN Biomedicals, Irvine, Calif.) as well as glucose (1 g/liter) and a mixture of 20 l-amino acids (Sigma Chemical Co., St. Louis, Mo.) (9) in Krebs-Henseleit bicarbonate (KHb) buffer (10). This perfusing medium was aerated with humidified O2·CO2 (95:5), and the pH was maintained at 7.4. The perfusate volume was 100 ml.

Experimental protocol. Eighteen rats in the following four treatment groups were studied: controls, no AII, no cefonicid (n = 5); AII, no drug (n = 4); cefonicid, no AII (n = 4); and cefonicid plus AII (n = 5). Cefonicid disodium (Smith Kline & French Laboratories, Philadelphia, Pa.) was dissolved in KHb (0.676 g of cefonicid per liter) for the loading dose and for the constant-rate infusion; cefonicid was added into the reservoir as a 5- to 10-μg/min infusion (7.5 to 15 μl/min). [14C]Inulin (16.7 μCi/ml; specific activity, 2.0 μCi/mg; ICN Radiochemicals, Irvine, Calif.) was dissolved in distilled water. All was dissolved in 0.9% NaCl and was introduced into the reservoir as a 2- to 5-ng/min infusion (13 to 32 μl/min).

Following a 15-min period of equilibration, 0.740 ml of cefonicid solution and 0.150 ml of [14C]inulin were introduced as boluses into the recirculating perfusion medium.

A major factor in the ability of an organ to remove a compound from the circulation is the rate at which the compound is delivered to the organ. The effect of flow rate on this drug removal process is critical, especially for those drugs in which the difference between the arterial and venous concentrations across the organ is very high (i.e., drugs with a high extraction ratio). However, the ability of disease to cause blood flow-induced changes in the hepatic clearance of low-extraction drugs has also been established (19, 22). Hence, it is relevant to consider whether the relationships between clearance and organ flow that operate for drug elimination from the liver apply as well for renal drug elimination.

The utility of the isolated perfused rat kidney in the evaluation of the disposition of compounds has been shown previously (3, 21). Isolated organs enable the study of renal function under states in which variables can be carefully and systematically changed (13, 14). Cefonicid, an expanded-spectrum cephalosporin, is 96% bound in humans (at 100 μg/mL), is actively secreted, and is neither metabolized nor reabsorbed in the isolated kidney (20a). In addition, cefonicid is a compound of low renal extraction in the isolated kidney as well as in humans. Angiotensin II (AII) is a strong vasoconstrictor hormone of the afferent and the efferent arterioles in the kidney (8). The decrease in perfusate flow by AII in the isolated kidney is well documented (8, 11, 15–17). We therefore wanted to establish the effects of changes in organ perfusion on the clearance parameters of cefonicid in isolated perfused rat kidneys.

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The cefonicid and AII infusions were started at this time. An additional 15 min was then allowed for drug distribution and hemodynamic stability to occur. The subsequent experimental time was divided into 10-min urine collection periods for the evaluation of physiological and pharmacokinetic parameters. The urine volume was measured, and its pH was determined immediately. Perfusate (1.5 ml) was sampled at the midpoint time of each clearance period. Losses in perfusate and urine because of sampling were minimized by isovolumetric replacement with blank perfusate and KHB buffer, respectively.

Analytical methods. Perfusate and urine samples were analyzed for [14C]insulin and glucose to assess the viability of the preparation. Inulin was assayed on an LS 5000TD liquid scintillation counter (Beckman Instruments, Irvine, Calif.) that uses an external standard method for quench correction. Glucose was determined with a YSI model 27 industrial analyzer (Fisher Scientific, Chicago, Ill.), which uses an immobilized glucose oxidase membrane connected to an electrochemical sensor.

Samples of perfusate and urine containing cefonicid were analyzed by the reversed-phase ion-pair liquid chromatographic technique of Phelps et al. (20), with minor modifications (20a). The cefonicid assay was linear over the perfusate concentration range of 0.5 to 200 µg/ml and over the urine concentration range of 8 to 800 µg/ml. A sensitivity limit of 0.5 µg/ml was achieved in the perfusate by using a sample volume of 100 µl and an injection volume of 50 µl. The perfusate and urine assays were validated by running three samples at low (2 µg/ml for perfusate, 8 µg/ml for urine), medium (50 µg/ml for perfusate, 400 µg/ml for urine), and high (200 µg/ml for perfusate, 800 µg/ml for urine) concentrations of cefonicid, both within a day and between 3 days. In all instances, the coefficient of variation was less than 15% and the bias was less than 10%.

Protein binding. The binding of cefonicid to albumin in the perfusate was measured by ultrafiltration, as reported previously (20a). The concentration of cefonicid in the dialysate was determined by the high-performance liquid chromatographic assay described elsewhere (20a). Preliminary studies (data not shown) ruled out membrane binding and albumin leakage and ensured drug stability during the ultrafiltration process. Therefore, the unbound fraction (fu) of cefonicid was calculated as the ratio of the concentration in the dialysate to that in the perfusate.

Data analysis. The renal clearances (CLR) of inulin, glucose, and cefonicid were calculated according to the equation: 

\[
\text{CLR} = \frac{U \times V}{C_{\text{mid}}},
\]

where \(U\) and \(C_{\text{mid}}\) are the substrate concentrations in the urine and perfusate, respectively, and \(V\) is the urinary flow rate. The fractional excretion of glucose (FEglycose) was calculated as 

\[
\text{FEglycose} = \frac{\text{CLR}_{\text{glucose}}}{\text{GFR}}.
\]

where \(\text{CLR}_{\text{glucose}}\) of inulin was taken to represent the glomerular filtration rate (GFR). The ratio of the perfusion pressure to the perfusate flow rate was taken to represent the renal vascular resistance of the isolated perfused rat kidney preparation. The results were expressed as the means ± standard errors of perfusion experiments in each treatment group. Each perfusion consisted of seven to nine urine collection periods. For tests to statistically significant differences among groups, two-sample t tests or two-way analyses of variance were performed when appropriate. Scheffe’s method of multiple comparisons was used if the F ratio was significant. For all tests, a significance level of \(\alpha = 0.05\) was used.

RESULTS AND DISCUSSION

Studies that have evaluated the effects of alterations in renal blood flow on the CLR of drugs are scarce (4, 6, 11), creating a rather descriptive approach to the analysis of the role of blood flow on renal drug excretion. Numerous physiological and pathophysiological conditions can affect blood flow through organs. For instance, cardiac failure causes the redistribution of blood flow, with disproportionately higher proportions of cardiac output reaching the brain and heart and smaller fractions perfusing the kidney, skin, and splanchnic tissues (4). Andreasen and Mikkelsen (1) reported an increase in the CLR of furosemide (from 39 to 77 ml/min) from the first to the second 1-h period after drug injection when it was administered to patients with left-sided heart failure. No changes in CLR were observed in normal subjects (from 116 to 117 ml/min). The investigators concluded that this increase may be due to a furosemide-induced amelioration in renal circulation, enhancing the tubular secretion of the drug. Furthermore, since furosemide is a compound of low renal extraction, this finding indicates that the renal excretion of furosemide could be influenced by a change in organ perfusion. A reduced CLR of furosemide

| TABLE 1. Physiological parameters of the isolated perfused rat kidneya |
|-----------------|--------------|-------------|-------------|---------|-------------|-------------|-------------|
| Treatment       | Perfusate flow rate (ml/min) | Perfusion pressure (mm Hg) | RVR (mm Hg - min/ml) | GFR (ml/min) | FF | FEglycose | Urine flow (ml/min) | Urine pH |
| No cefonicid    | 42.8 ± 1.4*  | 94 ± 2   | 6.23 ± 0.10  | 0.673 ± 0.046 | 0.016 ± 0.001 | 0.033 ± 0.007 | 0.109 ± 0.018 | 6.82 ± 0.06 |
| Without AII (n = 5) | 21.9 ± 0.9a | 96 ± 3   | 4.58 ± 0.29a | 0.460 ± 0.064 | 0.021 ± 0.003 | 0.050 ± 0.004 | 0.071 ± 0.016 | 6.59 ± 0.09 |
| With cefonicid  | 32.8 ± 3.4t  | 97 ± 3   | 3.15 ± 0.45t | 0.595 ± 0.054 | 0.019 ± 0.002 | 0.050 ± 0.009 | 0.061 ± 0.002 | 6.64 ± 0.10 |
| Without AII (n = 5) | 22.2 ± 0.5  | 95 ± 2   | 4.35 ± 0.21  | 0.608 ± 0.062 | 0.028 ± 0.003 | 0.035 ± 0.003 | 0.067 ± 0.003 | 5.33 ± 0.06 |
| With AII (n = 5)  |                |          |              | 0.0001 | 0.0001 | 0.1119 | 0.0088 | 0.1231 | 0.0769 | 0.0719 |
| P valueb         | 0.0004       | 0.0009   | 0.0001       | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

a Abbreviations: RVR, renal vascular resistance; GFR, glomerular filtration rate; FF, filtration fraction; FEglycose, fractional excretion of glucose. Symbols indicate significant pairwise contrasts at the 5% level for each column. Values are means ± standard errors.

b Significance values of overall F test from two-way analysis of variance.
was also observed by Brater et al. (7) in patients with congestive heart failure and in patients with other pathophysiological conditions (2) where renal blood flow was reduced. Still, an association between changes in renal perfusion and alterations in furosemide secretion is vague because renal blood flow was measured in only one study and the effect of differences in protein binding and renal function (GFR, FEglucose) were not judged in the analysis of the data.

The relationship between AII-induced flow changes and cefonicid clearance was examined in the study described here as a means to investigate whether the clearance of low-extraction compounds could be influenced by perturbations in organ flow. At uniform perfusion pressures among the experiments, no significant differences in functional nephron mass (GFR) and tubular transport (FEglucose) were observed as a function of either cefonicid or AII (Table 1). The renal tubular transport of the functioning nephrons was well preserved, as confirmed by the low and steady values of FEglucose. In contrast, there were expected and dramatic differences in the hemodynamics of the isolated kidney in the presence of AII. These differences included a 40% decrease in overall perfusate flow rate and increases of 40 and 65% in overall filtration fraction and renal vascular resistance, respectively. The clearance parameters of cefonicid were also reduced by approximately 40% in the presence of AII, with little or no change in protein binding (Table 2). This reduction occurred at similar total drug concentrations. Likewise, CLuR, CLuS, and ER were attenuated by 40 to 50% when the perfusate flow was reduced (Table 3); the unbound drug concentrations in these studies were essentially equivalent. Overall, this indicates that AII-induced perturbations in perfusate flow rate can impair the renal tubular transport of cefonicid in the isolated kidney.

The relationship between renal clearance and physiological determinants can be expressed, under linear conditions, as (12) \( CL_R = f_u \cdot GFR + Q_R \cdot CLu_{int,S} / (Q_R + f_u \cdot CLu_{int,S}) \), where \( Q_R \) is the renal plasma flow perfusing the secretory sites and \( CLu_{int,S} \) is the unbound intrinsic clearance of secretion; reabsorption is assumed to be nonexistent. In the case of low-extraction compounds, this equation simplifies to \( CL_R = f_u \cdot GFR + f_u \cdot CLu_{int,S} \), since \( Q_R \gg f_u \cdot GFR \). For highly secreted compounds, \( CL_R \) approximates \( CL_S = f_u \cdot CLu_{int,S} \), since \( f_u \cdot CLu_{int,S} \gg f_u \cdot GFR \). The excretion ratio (ER) can be expressed as \( ER = 1 + CLu_{int,S} / GFR \). Hence, a decrease in perfusate flow rate should not affect the \( CL_R \) or the ER of cefonicid unless the total number of nephrons (as reflected by GFR) and/or the efficiency of tubular secretion per unit nephron (CLu_{int,S})/GFR also decreases. In fact, a reduced renal perfusion may trigger tissular hypoxia or intrarenal redistribution of perfusate flow, and as a consequence, it may affect the ability of the kidney to secrete drug. Since the GFR was unchanged in cefonicid perfusions, with and without AII (Table 1), and since cefonicid is not reabsorbed (20a), the data are consistent with a flow-induced change in the CLuS or CLu_{int,S} of the drug.

In conclusion, results of these studies with cefonicid in the isolated perfused rat kidney demonstrate that a reduction in organ perfusion can result in notable decreases in the CLuR and CLuS of a low-extraction drug. This appears to be compatible with a reduced efficiency of tubular drug transport and may reflect perturbations other than those of perfusion flow rate alone.

**ACKNOWLEDGMENTS**

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**REFERENCES**


**TABLE 2. Pharmacokinetic parameters of total cefonicid in the isolated perfused rat kidney**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total concn (μg/ml)</th>
<th>( f_u )</th>
<th>CLuR (ml/min)</th>
<th>CLuS (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without AII (n = 4)</td>
<td>6.83 ± 0.80</td>
<td>0.284 ± 0.019</td>
<td>1.63 ± 0.12</td>
<td>1.46 ± 0.10</td>
</tr>
<tr>
<td>With AII (n = 5)</td>
<td>5.22 ± 1.45</td>
<td>0.321 ± 0.016</td>
<td>1.03 ± 0.17</td>
<td>0.835 ± 0.143</td>
</tr>
<tr>
<td>( P ) value</td>
<td>0.3983</td>
<td>0.1683</td>
<td>0.0162</td>
<td>0.0126</td>
</tr>
</tbody>
</table>

* Values are means ± standard errors.

\( \text{a} \) Significance values correspond to two-sample \( t \) tests.

**TABLE 3. Pharmacokinetic parameters of unbound cefonicid in the isolated perfused rat kidney**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Unbound concn (μg/ml)</th>
<th>CLuR (ml/min)</th>
<th>ER</th>
<th>CLuS (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without AII (n = 4)</td>
<td>1.86 ± 0.16</td>
<td>6.07 ± 0.78</td>
<td>10.3 ± 1.0</td>
<td>5.41 ± 0.69</td>
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<tr>
<td>With AII (n = 5)</td>
<td>1.71 ± 0.50</td>
<td>3.50 ± 0.71</td>
<td>6.03 ± 1.39</td>
<td>2.89 ± 0.71</td>
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<tr>
<td>( P ) value</td>
<td>0.8401</td>
<td>0.0458</td>
<td>0.0486</td>
<td>0.0418</td>
</tr>
</tbody>
</table>

* Values are means ± standard errors.

\( \text{a} \) Significance values correspond to two-sample \( t \) tests.


