Nephrotoxicity of Acyclovir and Ganciclovir in Rats: Evaluation of Glomerular Hemodynamics

MARIA DE FATIMA FERNANDES DOS SANTOS,* OSCAR FERNANDO PAVÃO DOS SANTOS,* MIRIAN APARECIDA BOIM,* CLARA VERSOLATO RAZVICKAS,* LUIS ANTONIO RIBEIRO DE MOURA,† HORÁCIO AJZEN,* and NESTOR SCHOR*

*Nephrology Division and †Pathology Department, Escola Paulista de Medicina, São Paulo, Brazil.

Abstract. Whole-kidney function and glomerular hemodynamics were evaluated after acute (50 mg/kg, iv, in bolus) and short-term chronic (50 mg mg/kg, ip, 5 days) acyclovir (ACV) and short-term chronic ganciclovir (Gan; 30 mg/kg, ip, 5 days) treatment in euvolemic Munich-Wistar rats. The evaluation of whole-kidney function of the ACV groups showed a significant reduction in total GFR (0.96 ± 0.10 to 0.28 ± 0.02 mL/min in the acute group, P < 0.05, and 1.04 ± 0.09 to 0.33 ± 0.04 mL/min in the chronic group, P < 0.05) with a marked increase in total renal vascular resistance (TRVR) (33 ± 5 to 122 ± 26 mm Hg · min/mL in the acute group and 28 ± 3 to 74 ± 18 mm Hg · min/mL in the chronic group, P < 0.05) and a reduction in RPF (2.29 ± 0.25 to 0.81 ± 0.15 mL/min in the acute group and 2.57 ± 0.36 to 1.30 ± 0.40 mL/min in the chronic group, P < 0.05). Conversely, urinary flow (V′) was unchanged (3.6 ± 0.4 to 3.6 ± 0.2 µL/min in the acute group) or elevated (3.7 ± 0.6 to 6.6 ± 1.4 µL/min in the chronic group, P < 0.05). The evaluation of glomerular hemodynamics after ACV treatment showed a reduction in single-nephron GFR (SNGFR) (46.4 ± 5.3 to 26.2 ± 3.4 mL/min in the acute group and 38.7 ± 5.7 to 21.1 ± 5.7 mL/min in the chronic group, P < 0.05), a significant elevation in total arteriolar resistance (Rt) (2.90 ± 0.44 to 4.94 ± 0.77 × 10^10 dyn · s · cm^{-5} in the acute group and 3.72 ± 0.45 to 9.00 ± 2.40 × 10^{10} dyn · s · cm^{-5} in the chronic group, P < 0.05) and a severe reduction in glomerular plasma flow rate (QA) (152.6 ± 29.5 to 103.8 ± 27.8 mL/min in the acute group and 149.1 ± 29.8 to 68.5 ± 10.0 mL/min in the chronic group, P < 0.05). However, the glomerular ultrafiltration coefficient, Kf, was changed only in the chronic group (0.1002 ± 0.0165 to 0.0499 ± 0.0090 mL/(s · mm Hg), P < 0.05). After Gan treatment, no changes were observed in GFR (1.04 ± 0.09 to 0.96 ± 0.08 mL/min, with the maintenance of RPF (2.57 ± 0.36 to 2.66 ± 0.34 mL/min) and a nonsignificant reduction in TRVR (28 ± 3 to 20 ± 3 mm Hg · min/mL). The short-term Gan treatment also showed a different pattern in glomerular hemodynamics by inducing an elevation in SNGFR (38.7 ± 5.7 to 50.3 ± 2.8 mL/min, P < 0.05) with no changes in QA (150 ± 30 to 135 ± 22 mL/min) and a mild vasodilation, Rt (3.7 ± 0.5 to 2.7 ± 0.3 × 10^{10} dyn · s · cm^{-5}, P < 0.05) associated with an increment in Kf (0.1002 ± 0.0165 to 0.2400 ± 0.0700 mL/(s · mm Hg), P < 0.05). Thus, ACV induced acute renal failure by reducing GFR and SNGFR by an increase in TRVR and Rt with a reduction in RPF and QA. Also, after short-term treatment with ACV, a reduction in Kf led to a reduction of SNGFR. On the other hand, Gan treatment did not induce acute renal failure by the adopted techniques. (J Am Soc Nephrol 8: 361–367, 1997)

In fact, herpes and cytomegalovirus infections are a major cause of morbidity and mortality in patients with AIDS (2). Also, cytomegalovirus is well recognized as the most important pathogen in recipients of bone marrow (5) and renal transplants (6), and it is known to be responsible for more than 90% of viral infection recurrence in AIDS patients (7,8).

On this basis, the potential side-effects of these compounds are a source of concern in medical practice, and many studies about ACV and some about Gan toxicity have been conducted by several investigators. Effects such as neurotoxicity and nephrotoxicity have already been reported after ACV treatment, but renal damage is the most common and speculative side-effect of this antiviral drug (9). Gan seems to be responsible for neutropenia in 30% of patients, but no evidence of renal damage has been associated with this compound (9). The possibility that Gan is not nephrotoxic, and its potential use in effective antiviral drug therapy is currently available, especially in the area of herpes infections. The use of acyclovir (ACV) has become an established part of clinical practice, and other compounds such as ganciclovir (Gan) an ACV-like drug, are being increasingly used in the treatment of herpes simplex and cytomegalovirus diseases in immunocompromised hosts. Thus, because vaccines for these pathogens are still being developed, antiviral agents will continue to play a major role in the management of all virus infections (1–4).
the management of other viral infections, encourages its use as an alternative to ACV in cases of renal failure, and stimulated this study on a comparison of their effects on renal function.

In fact, the presumed mechanism of ACV-induced renal damage was first demonstrated by animal studies. Rats that received intravenous ACV (20 mg/kg per d) developed an obstructive nephropathy secondary to drug crystal formation in collecting ducts (10,11). Crystaluria was also observed to be dose-dependent and reversible, depending on the drug discontinuation. Studies performed in mice receiving single 50-mg ip doses of ACV demonstrated crystal formation in collecting tubules, with gradual diminution in refractile material over doses of ACV (12). After only 20 tubules, with gradual diminution in refractile material over time between the experiment and the morphological studies biopsy of a patient affected by ACV nephropathy (14). Moreover, crystal dissolution after the fixation methods used and the time between the experiment and the morphological studies must be taken into consideration, because they may eliminate the obstruction mechanism of ACV nephropathy. However, no glomerular effect has been demonstrated on the level of renal microcirculation when ACV is given in a dose (acute or chronic) that does not cause crystalluria, crystal deposition, or tubular obstruction.

Other animal studies about different nephrotoxic agents have also demonstrated severe glomerular hemodynamic alterations without important renal morphology changes (e.g., gentamicin, cisplatin), suggesting that the development of acute renal failure (ARP) in these experimental models may be of functional origin (15,16).

Thus, to investigate the physiology of the renal impairment induced by ACV in the absence of crystalluria, and the possible renal events after Gan treatment, groups of rats submitted to acute or chronic short-term treatment with ACV or to chronic short-term treatment with Gan were evaluated. Also, a group of rats receiving only vehicle was used as a control. Whole renal function and glomerular hemodynamics were evaluated in all animals. In addition, pathologic analysis of kidney specimens was carried out in the ACV group.

Materials and Methods

Studies were performed in four groups of adult male Munich-Wistar rats weighing 250 to 300 g. The first group (N = 11, acute ACV) after a period of equilibration received an intravenous injection of ACV, 50 mg/kg body wt in bolus (3 min infusion) in a 0.1-mL volume. The second group (N = 10, chronic ACV) was treated with ACV 50 mg/kg body wt, ip, daily for 5 days. The third group (N = 9) was also treated daily for 5 days with ACV-vehicle (saline), 0.1 mL. The fourth group received chronic Gan treatment, 30 mg/kg body wt, ip, daily for 5 days. All rats were allowed free access to water and a standard rat-pellet diet until the morning of the study. Rats were anesthetized with Inactin (BYK, Konstanz, Germany), 100 mg/kg, ip, placed on a temperature-regulated table, and monitored with an electronic rectal thermometer (Simpson Co., Chicago, IL) to keep body temperature between 37 and 37.5°C. After the rats underwent tracheotomy, the left femoral artery was catheterized with PE-50 polyethylene tubing, and approximately 60 μL of arterial blood was collected for baseline hematocrit (Hct) and protein (CaO) determination.

The same arterial catheter was also used for subsequent periodic blood sampling and evaluation of mean femoral arterial pressure (MAP) with an electronic transducer (Model P23Db; Statham Instruments Div., Gould Inc., Hato Rey, Puerto Rico) connected to a direct-writing recorder (Model 2.200; Gould Inc., Cleveland, Ohio). Polyethylene catheters were also introduced into the right and left jugular veins for infusion of inulin, paraaminohippuric acid (PAH), and isoncotic rat serum. The animals were then infused intravenously with 10% inulin and a 2% PAH solution in 0.9% sodium chloride at a rate of 1.2 mL/h. Isoncotic rat serum was infused into the left vein to replace surgical losses and thus maintain the animals in euvolemic conditions (14). Laparotomy was then performed, and a PE-10 polyethylene catheter was introduced into the left ureter for collection of timed urine samples, after which rats were routinely prepared for micropuncture studies (17–19).

Micropuncture Studies

Glomerular hemodynamic evaluations were done by micropuncture technique. Exactly timed (1 to 3 min) samples of tubular fluid were collected from surface convolutions of at least three nephrons for determination of flow rate and inulin concentration, and calculation of single-nephron GFR (SNGFR). Simultaneously, two or three samples of femoral arterial blood were obtained during each period for determination of systemic arterial pressure, Hct, total protein, inulin, and PAH concentration in plasma.

To estimate the colloidal osmotic pressure of plasma entering and leaving glomerular capillaries, we measured protein concentrations in efferent arteriole (Cpe) blood plasma, as previously described (21). Cα (in the femoral artery) was taken as a parameter of protein concentration in the efferent arteriole. At the same time, hydraulic pressures were measured in the superficial structures of the kidneys by a continuous recording system (IPM Inc., San Diego, CA). Micropipettes with outer tip diameters of 2 to 4 μm and containing 2.0 M sodium chloride were used. Hydraulic pressure output from the servosystem was channeled via an electronic transducer (Statham model P23Db) to a second channel of the recorder. Direct measurements of hydraulic pressure in single glomerular capillaries (Pocc), proximal tubules (Pip), efferent arterioles (Preα), and third-order peritubular capillaries (Pc) were recorded in each rat.

At the end of the functional studies, kidney samples were fixed in a solution containing 2% glutaraldehyde, Bouin, Methanol, or Dubosch.

Analytical Procedures

The concentration of inulin in tubular fluid was determined, usually in duplicate, by the microfluorescence method of Vurek and Pegram (22). Inulin and PAH concentrations in plasma and urine were obtained by the macroanthrone method of Fuhr et al. (23) and by the method of Smith et al. (24), respectively. Protein concentration in the femoral artery was determined by refractometry and corrected according to a curve constructed by the method of Lowry and modified by
Glomerular filtration rate was evaluated by inulin clearance, and RPF by PAH clearance. Total renal vascular resistance (TRVR) was estimated as the MAP/RPF ratio. The filtration fraction (FF) was calculated as the quotient of GFR and RPF. Values of GFR and RPF were corrected for kidney weight.

Colloidal osmotic pressures, hydraulic pressures, and SNGFR allow the calculation of single-nephron filtration fraction (SNFF), initial glomerular capillary plasma flow rate (QG), afferent (RA), efferent (RE), and total (RT = RA + RE) arteriolar resistances, and glomerular ultrafiltration coefficient (Kf), using equations given elsewhere (25).

Statistical Analysis
Data were analyzed statistically by the paired and unpaired t test, with the level of statistical significance set at \( P < 0.05 \). All data are reported as mean \( \pm \) SE.

Results
Tables 1 and 2 summarize the results of general data and whole-kidney function for the acute and chronic ACV groups. Initial body weight (BW\(_{\text{in}}\)), kidney weight (KW), MAP, initial and final hematocrit (Hct\(_{\text{in}}\) and Hct\(_{\text{f}}\)), and initial and final afferent protein concentration (C\(_{A0}\) and C\(_{A}\)) did not differ between groups.

Nonoliguric ARF was detected on the basis of urine output after acute intravenous infusion of ACV (3.6 \( \pm \) 0.4 to 3.6 \( \pm \) 0.2 \( \mu \)L/min). Moreover, GFR was hardly decreased (0.96 \( \pm \) 0.10 to 0.28 \( \pm \) 0.02 mL/min, \( P < 0.05 \)) because of severe increase in TRVR (33 \( \pm \) 5 to 122 \( \pm \) 26 mm Hg \( \cdot \) min/mL, \( P < 0.05 \)) which also determined a decline in RPF (2.29 \( \pm \) 0.25 to 0.81 \( \pm \) 0.15 mL/min, \( P < 0.05 \)). This proportional decrease in GFR and RPF maintained FF at levels that were unchanged (43 \( \pm \) 1 to 38 \( \pm \) 1%) (Table 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>KW (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>Hct(_{\text{in}}) (%)</th>
<th>Hct (g/dL)</th>
<th>C(_{A0}) (g/dL)</th>
<th>C(_A) (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-acute ACV</td>
<td>261 ± 7</td>
<td>1.4 ± 0.1</td>
<td>130 ± 4</td>
<td>45 ± 1</td>
<td>46 ± 1</td>
<td>5.1 ± 0.1</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>Post-acute ACV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Summary of general data for the acute acyclovir (ACV)* group

* Values shown are means \( \pm \) SE. BW, body weight; KW, kidney weight; MAP, mean arterial pressure; Hct, hematocrit, C\(_A\), afferent protein.

b The subscript 0 refers to baseline values at the beginning of anesthesia.
Table 2. Summary of general data for the chronic ACV group\textsuperscript{a}

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BW\textsuperscript{b} (g)</th>
<th>KW (g)</th>
<th>MAP (mm Hg)</th>
<th>Hct\textsubscript{r} (%)</th>
<th>Hct</th>
<th>C\textsubscript{AO} (g/dL)</th>
<th>C\textsubscript{A} (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>250 ± 6</td>
<td>1.0 ± 0.4</td>
<td>138 ± 5</td>
<td>45 ± 1</td>
<td>5.1 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Chronic</td>
<td>265 ± 6</td>
<td>1.1 ± 0.1</td>
<td>132 ± 6</td>
<td>46 ± 1</td>
<td>5.2 ± 0.1</td>
<td>5.3 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values shown are means ± SE. Abbreviations are defined in the footnote to Table 1.
\textsuperscript{b} in, initial body wt; fn, final body wt.
\textsuperscript{c} \(P < 0.05\), final versus initial body weight.

Table 3. Summary of whole-kidney function data for the acute ACV group\textsuperscript{a}

<table>
<thead>
<tr>
<th>Group</th>
<th>V\textsuperscript{'} (μL/min)</th>
<th>GFR (mL/min)</th>
<th>RPF (mL/min/mL)</th>
<th>FF (%)</th>
<th>TRVR (mm Hg·min/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-acute ACV</td>
<td>3.6 ± 0.4</td>
<td>0.96 ± 0.10</td>
<td>2.29 ± 0.25</td>
<td>43 ± 1</td>
<td>33 ± 5</td>
</tr>
<tr>
<td>Post-acute ACV</td>
<td>3.6 ± 0.2</td>
<td>0.28 ± 0.02\textsuperscript{b}</td>
<td>0.81 ± 0.15\textsuperscript{b}</td>
<td>38 ± 1</td>
<td>122 ± 26\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values shown are means ± SE. V\textsuperscript{'} , urinary flow; FF, filtration fraction; TRVR, total renal vascular resistance.
\textsuperscript{b} \(P < 0.05\), pre-acute ACV versus post-acute ACV.

Table 4. Glomerular hemodynamic measurements for the acute ACV group\textsuperscript{a}

<table>
<thead>
<tr>
<th>Group</th>
<th>SNGFR (nL/min)</th>
<th>QA (nL/min)</th>
<th>SNFF (%)</th>
<th>P\textsubscript{GC} (mm Hg)</th>
<th>P\textsubscript{T} (mm Hg)</th>
<th>ΔP (mm Hg)</th>
<th>P\textsubscript{EA} (mm Hg dyn·s·cm\textsuperscript{-5})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-acute ACV</td>
<td>46.4 ± 5.3</td>
<td>152.6 ± 29.5</td>
<td>34 ± 1</td>
<td>44 ± 2</td>
<td>16 ± 1</td>
<td>28 ± 2</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>Post-acute ACV</td>
<td>26.2 ± 3.4\textsuperscript{b}</td>
<td>103.8 ± 27.8\textsuperscript{b}</td>
<td>31 ± 1</td>
<td>42 ± 2</td>
<td>18 ± 1</td>
<td>24 ± 1</td>
<td>22 ± 1</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values shown are means ± SE. SNGFR, single-nephron GFR; QA, glomerular plasma flow rate; SNFF, single-nephron filtration fraction; P\textsubscript{GC}, single glomerular capillary pressure; P\textsubscript{T}, proximal tubule pressure; ΔP, transcapillary hydraulic pressure gradient; P\textsubscript{EA}, efferent arteriolar pressure.

\textsuperscript{b} \(P < 0.005\), post-acute ACV versus pre-acute ACV.

Table 5. Glomerular hemodynamic data of acute ACV group\textsuperscript{a}

<table>
<thead>
<tr>
<th>Group</th>
<th>RA (× 10\textsuperscript{-10} dyn·s·cm\textsuperscript{-5})</th>
<th>RE (g/dl)</th>
<th>RT (nL/(s·mm Hg))</th>
<th>CA (g/dl)</th>
<th>CE (nL/(s·mm Hg))</th>
<th>Eq/Des</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-acute ACV</td>
<td>1.90 ± 0.32</td>
<td>1.00 ± 0.15</td>
<td>2.90 ± 0.44</td>
<td>5.0 ± 0.1</td>
<td>7.7 ± 0.2</td>
<td>0.1149 ± 0.0200</td>
</tr>
<tr>
<td>Post-acute ACV</td>
<td>3.66 ± 0.63\textsuperscript{b}</td>
<td>1.29 ± 0.20\textsuperscript{b}</td>
<td>4.94 ± 0.77\textsuperscript{b}</td>
<td>5.0 ± 0.2</td>
<td>7.4 ± 0.2</td>
<td>0.1331 ± 0.0222</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values are shown as means ± SE. Eq/Des, equilibrium/disequilibrium ratio. All other abbreviations defined in preceding tables’ footnotes.

\textsuperscript{b} \(P < 0.05\), pre-acute ACV versus post-acute ACV.

10\textsuperscript{10} dyn·s·cm\textsuperscript{-5}, \(P < 0.05\) when compared with the reduction in RA (2.34 ± 0.36 to 2.12 ± 0.24 × 10\textsuperscript{10} dyn·s·cm\textsuperscript{-5}) induced a nonsignificant decrease in ΔP (31 ± 2 to 24 ± 2 mm Hg). SNFF was also unchanged (31 ± 1 to 38 ± 1%) (Figure 1).

Discussion
Precise data on the prevalence of herpes simplex virus (HSV) infections in immunocompromised individuals such as AIDS patients and allograft recipients are not available, but evidence suggests that the rate parallels or exceeds that of the general population (2). Also, the incidence of cytomegalovirus (CMV) infections in this population is high, and CMV is a major cause of morbidity and mortality in AIDS patients and in recipients of bone marrow or solid organ transplants (1). In fact, the effective specific antiviral chemotherapy for HSV and CMV is still represented by ACV and Ganc (3).

Thus, the main purpose of this study was to determine the possible damage of renal function induced by these two drugs by submitting Munich-Wistar rats to ACV (acute and chronic treatment) and ganciclovir (chronic treatment), and evaluating the overall renal function and glomerular hemodynamics of
Table 6. Summary of whole-kidney function data for the chronic ACV group

<table>
<thead>
<tr>
<th>Treatment</th>
<th>V (μL/min)</th>
<th>GFR (mL/min)</th>
<th>RPF (%/min)</th>
<th>FF (%)</th>
<th>TRVR (mm Hg · min/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>3.7 ± 0.6</td>
<td>1.04 ± 0.09</td>
<td>2.57 ± 0.36</td>
<td>43 ± 6</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>Chronic ACV</td>
<td>6.6b ± 1.4</td>
<td>0.33b ± 0.04</td>
<td>1.30b ± 0.40</td>
<td>40 ± 1</td>
<td>74b ± 18</td>
</tr>
</tbody>
</table>

* Values shown are means ± SE. V, volume; TRVR, total renal vascular resistance. All other abbreviations defined in preceding tables' footnotes.

b P < 0.05, chronic ACV versus vehicle.

Table 7. Glomerular hemodynamic data for vehicle and chronic ACV groups

<table>
<thead>
<tr>
<th>Group</th>
<th>SNGFR (nL/min)</th>
<th>QA (L/min)</th>
<th>SNFF (%)</th>
<th>Pgc (mm Hg)</th>
<th>Pt (mm Hg)</th>
<th>ΔP (mm Hg)</th>
<th>Pea (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>38.7 ± 5.7</td>
<td>149.1 ± 29.8</td>
<td>31 ± 1</td>
<td>45 ± 2</td>
<td>14 ± 1</td>
<td>31 ± 1</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>Chronic ACV</td>
<td>21.1 ± 7.7b</td>
<td>68.5 ± 10.1b</td>
<td>31 ± 1</td>
<td>55 ± 3b</td>
<td>24 ± 4b</td>
<td>31 ± 2</td>
<td>23 ± 2</td>
</tr>
</tbody>
</table>

Values shown are means ± SE. All abbreviations are defined in preceding tables' footnotes.

b P < 0.05, chronic ACV versus vehicle.

Table 8. Glomerular hemodynamic data for the chronic ACV group

<table>
<thead>
<tr>
<th>Group</th>
<th>RA (× 10¹⁰ dyn · cm⁻²)</th>
<th>RE (× 10¹⁰ dyn · cm⁻²)</th>
<th>RT (× 10¹⁰ dyn · cm⁻²)</th>
<th>CA (g/dL)</th>
<th>CE (g/dL)</th>
<th>Kt (L/s · cm⁻² · mm Hg⁻¹)</th>
<th>Eq/Des</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>2.34 ± 0.36</td>
<td>1.18 ± 0.16</td>
<td>3.72 ± 0.45</td>
<td>5.1 ± 0.1</td>
<td>7.7 ± 0.3</td>
<td>0.1002 ± 0.0165</td>
<td>7/3</td>
</tr>
<tr>
<td>Chronic ACV</td>
<td>4.10 ± 0.80b</td>
<td>4.90 ± 0.90b</td>
<td>9.00 ± 2.40b</td>
<td>5.3 ± 0.1b</td>
<td>7.7 ± 0.3</td>
<td>0.0499 ± 0.0090b</td>
<td>6/4</td>
</tr>
</tbody>
</table>

* Values shown are means ± SE. All abbreviations are defined in preceding tables' footnotes.

b P < 0.05, chronic ACV versus vehicle.

these animals by clearance and micropuncture techniques. A pilot study was performed (data not shown) to determine an ACV dose that did not cause crystalluria or tubular obstruction. In fact, only ACV has been associated with episodes of ARF in patients submitted to large dosages and long-term treatment (26), but the widespread clinical use of Gan led us to study the side-effects of this drug also.

ACV promoted ARF, with a reduction in GFR, marked vasoconstriction, and maintenance or elevation of urine output. Previous clinical observations showed increased urinary β2-microglobulin excretion in a patient submitted to ACV treatment, with no changes in serum creatinine. Therefore, because this protein is filtered and reabsorbed by the proximal tubule, this finding can indicate a probable ACV toxicity to this segment of the nephron (27). In fact, microperfusion studies with rats treated with ACV showed that the diffusional water permeability in the inner medullary collecting duct did not increase significantly when vasopressin was added to the bath, as observed in the tubules of normal rats (28). In the study presented here, the high urine output and the significant increase in P_t are in accordance with these observations, and together suggest that a tubular defect in the handling of antidiuretic hormone is really involved in this ARF.

The glomerular hemodynamic studies of acute and chronic ACV groups presented in this article demonstrated a reduction in SNGFR. The reduced SNGFR observed in acute ACV-treated animals occurred as a result of a decline in QA. On the other hand, the decline in SNGFR after short-term chronic ACV treatment was not only the result of a reduction in QA, but also of an important decrease in K_t. These effects on QA reduction in both kinds of treatment with ACV may have been caused by severe vasoconstriction, as demonstrated by the elevations in R_a and R_e. These increments were probably mediated by hormonal actions. However, because no hormonal blockade was used in this study, we cannot conclude which hormonal system is really involved in this ARF.

However, the apparent reduced ΔP observed in this study, when compared with the usual mean in the literature, cannot be responsible for the decrement in SNGFR because it is in accordance with our laboratory pattern for this parameter under control conditions, as already demonstrated by Barros et al. (30,31), Santos et al. (17), and Haddad et al. (32).

Kidney samples from animals of the ACV groups were analyzed for morphology by light microscopy techniques, and no alteration was observed in glomerular or tubular morphology by this method, even after four different methods of
Figure 1. Percentage changes in overall function and glomerular hemodynamics in rats submitted to acute and chronic acyclovir and ganciclovir treatment. SNGFR, single-nephron GFR; TRVR, total renal vascular resistance; T_{A}, total arteriolar resistance; Q_{A}, glomerular plasma flow rate; ΔP, transcapillary hydraulic pressure gradient; K_{f}, glomerular ultrafiltration coefficient. * P < 0.05, acute versus pre-acute; ** P < 0.05, acyclovir versus saline; *** P < 0.05, ganciclovir versus saline. Filled bars, acute ACV; hatched bars, chronic ACV; dotted bars, chronic Gan.

fixation were used to reduce the possibility of dissolving tubular drug crystals.

In fact, the euvoletic condition of the rats submitted to this study, with the dose adopted, could have protected them from accumulating crystals in renal tubules, and no obstructive event was observed. Nevertheless, in our preliminary results (which are not presented here because they were determined from a solely dosage-managing study), much larger doses of ACV (up to 300 mg/kg) induced ureter obstruction, and ACV crystals were observed in this segment after an anuric period, which can emphasize a dose-dependent mechanism for this signal. Thus, this study’s model, with 50 mg/kg of ACV and the glomerular changes observed, could also precede the obstruction observed when larger doses are administered.

As has been observed for some other nephrotoxic agents (e.g., cisplatin, gentamicin, and cyclosporin A), a high dose of these drugs is necessary to induce ARF (16,17,30,31). Also, one should consider that the doses of ACV and Gan in this study were very large in comparison with those used in humans—in fact, it is almost three times larger. However, it is fundamental to reiterate that different patterns of drug resistance among species have been already extensively discussed (33,34), and in comparison with humans, the rat is much more resistant to nephrotoxic insults because of a larger proportion of kidney weight to body weight, and the relation of GFR/kidney weight is approximately three times that described for humans. This fact obligated our laboratory to choose the large dosage of each drug.

Moreover, the lack of correlation between functional and morphological injuries can also indicate that different pathogenic mechanisms are involved in acute and chronic ACV nephrotoxicity, as well as provide an explanation for the sudden decline of renal function sometimes observed after a first-time administration of ACV. On the other hand, the glomerular hemodynamic alterations caused by acute and chronic toxicity of ACV administration were found to be different. Thus, the role of vasoactive systems is probably modified during the evolution of these two models of ACV nephrotoxicity. The repetitive administration of ACV could cause a modification of glomerular response to this drug, leading to a more important mesangial cell contraction and consequent reduction on K_{f}. A similar glomerular hemodynamic pattern for different periods of administration has been also described for cyclosporine A nephrotoxicity (29,30).

In contrast, Gan treatment did not affect overall renal function or glomerular hemodynamics, indicating that this drug—at least in this model—had no side-effect on renal function, or that the dosage used did not cause any renal alterations, despite being higher than that used for humans. On the other hand, the increase in K_{f} observed in this group was responsible for a nonsignificant elevation on SNGFR, but the specific determinants involved in this alteration cannot be explained once this parameter has been calculated only from a differential equation that involves the changes in the velocity protein concentration in the whole glomerular capillary idealized by Deen et al. (26) and not measured. Exclusively, the real measurement of K_{f} could specify which determinant, whether the hydraulic conductivity or the available filtration area, has induced such a change in this coefficient.

In summary, this study has shown that ACV, at the dose used, induced a nonoliguric ARF and, in contrast to most studies about this drug, no tubular obstruction was detected in the animal’s kidneys by light microscopy. It is the first demonstration of ACV effects on renal microcirculation in the absence of crystalluria.

Thus, although the evaluation of which hormones are in-
volved in the renal disturbances observed after ACV administration was not the objective of this article, the sole glomerular hemodynamic evaluation can itself demonstrate the severe changes in the renal microcirculation that occur when the acute renal failure by ACV is installed. Indeed, the hormonal investigation, as well as cellular protocols, should really contribute to a better understanding of this nephrotoxicity. ACV, but not Gan, may cause renal damage, as shown by the overall renal function and glomerular hemodynamic data in these studies. If the substitution of ACV by Gan could be considered in some cases, this correlation between their different actions on renal function can lead to a more rational choice of antiviral drug to minimize the possible side effects frequently observed in the immunosuppressed patients who are usually in need of this kind of therapeutic treatment.

References
6. Deleted in proof.