Effects of Fosfomycin and Imipenem/Cilastatin on Nephrotoxicity and Renal Excretion of Vancomycin in Rats

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Purpose. The effects of fosfomycin and imipenem/cilastatin on the nephrotoxicity of vancomycin were studied in rats, and those on the renal handling of vancomycin were also investigated in perfused kidneys.

Methods. The protective effects of fosfomycin and imipenem/cilastatin on vancomycin nephrotoxicity were evaluated by increases in plasma concentration of creatinine and urea nitrogen in rats. The urinary excretion of vancomycin was measured and analyzed kinetically in the perfused rat kidney.

Results. The nephrotoxicity induced by vancomycin (500 mg/kg, i.v.) was inhibited almost completely by co-administration of fosfomycin or imipenem/cilastatin. In the perfused rat kidney, the excretion ratio of vancomycin was less than those of p-aminohippurate and creatinine, and greater than that of arbekacin, suggesting the secretion and reabsorption of vancomycin in renal tubules. The tissue/perfusate ratios of unbound vancomycin were not significantly changed by co-treatment with fosfomycin or imipenem/cilastatin. Imipenem/cilastatin significantly decreased the excretion ratio of vancomycin. Fosfomycin also decreased vancomycin excretion ratio, although this effect was not significant.

Conclusions. The renal handling of vancomycin was different from those of organic anions and cations and an aminoglycoside antibiotic. The protective effects of fosfomycin and imipenem/cilastatin against the nephrotoxicity of vancomycin might be partly due to the change in renal handling of vancomycin, probably in its tubular secretion/reabsorption, in rats.

KEY WORDS: vancomycin; fosfomycin; imipenem/cilastatin; nephrotoxicity; protective effect; renal handling.

INTRODUCTION

Vancomycin hydrochloride, a glycopeptide antibiotic, is frequently used to treat infections with methicillin-resistant staphylococci (1). However, the pharmacokinetics of vancomycin are significantly affected by changes in renal functions, and the antibiotic may have otoxicity and nephrotoxicity at high plasma concentrations (2). On the other hand, it was reported that antibiotics such as fosfomycin and imipenem/cilastatin may decrease the nephrotoxicity of vancomycin by inhibiting its uptake into the rabbit kidney (3). Therefore, co-administration of fosfomycin or imipenem/cilastatin with vancomycin was expected to reduce the nephrotoxicity associated with vancomycin. Vancomycin is eliminated mainly by the kidneys. We previously reported that vancomycin is secreted via renal tubules, and that quinidine decreases the total clearance of vancomycin in rats (4). However, the protective effects of fosfomycin and imipenem/cilastatin on vancomycin-induced nephrotoxicity and their influences on the renal handling of vancomycin have not been elucidated.

To confirm the protective effects of fosfomycin and imipenem/cilastatin, the impairment of the kidney induced by administration of vancomycin was evaluated by measuring the plasma concentrations of creatinine and urea nitrogen in rats. Furthermore, to understand the renal handling of vancomycin in detail, it is necessary to eliminate extrarenal effects. The urinary excretion of vancomycin was investigated and compared with those of p-aminohippurate, creatinine and arbekacin using the perfused rat kidney model with constant perfusion rate. The effects of quinidine, fosfomycin and imipenem/cilastatin on the urinary excretion of vancomycin were also examined. The results suggested that the renal handling of vancomycin is quite different from those of anionic or cationic drugs and an aminoglycoside antibiotic, and that the protective effects of fosfomycin and imipenem/cilastatin on vancomycin nephrotoxicity may be associated with reduction of the renal tubular transport of vancomycin.

MATERIALS AND METHODS

Materials

Vancomycin hydrochloride was obtained from Shionogi (Osaka, Japan). p-Aminohippuric acid sodium salt, creatinine, quinidine sulfate and creatinine were purchased from Nacalai Tesque (Kyoto, Japan). Fosfomycin sodium and arbekacin sulfate for injection were obtained from Meiji Seika Kaisha (Tokyo, Japan). Imipenem/cilastatin sodium for injection was obtained from Banyu (Tokyo, Japan). Bovine serum albumin was obtained from Miles Inc. (Illinois, USA). All other chemicals were of the highest purity available.

Animals

Male Wistar albino rats weighing 194–303 g and 271–325 g were used for these in vivo and perfused kidney studies, respectively. Animals were maintained in metabolic cages before the experiments with free access to food and water. The animal experiments were performed in accordance with the Guideline for Animal Experiments of Kyoto University.

Effects of Fosfomycin and Imipenem/Cilastatin on Vancomycin-induced Nephrotoxicity in Rats

Nephrotoxicity was induced by administration of vancomycin (300 or 500 mg/kg, i.v.). Normal healthy rats served as controls. Fosfomycin (300 mg/kg) or imipenem/cilastatin (150/150 mg/kg) was administered intravenously with 300 and 500 mg/kg of vancomycin. To determine the concentrations of creatinine and urea nitrogen in the plasma, blood was obtained two days after injection of the drugs.

Renal Handling of Vancomycin in Perfused Rat Kidneys

The rat kidney was perfused as described previously (5), with some modifications. Briefly, the animals were anesthetized...
with pentobarbital (50 mg/kg), and 100 mg of mannitol in isotonic saline was injected into the femoral vein. The right kidney was exposed, and the ureter was cannulated for urine collection using a PE-10 tube. Heparin solution (1000 IU/kg) was injected into the femoral vein, and a venous cannula (o.d. 2 mm, i.d. 0.8 mm) was placed in the vena cava just below the right renal vein. The renal artery was cannulated via the mesenteric artery using a 20 G needle, and the kidney was perfused without interrupting the renal blood flow. The rat kidney was equilibrated with constant perfusion at 16 ml/min. The perfusate, Krebs-Henseleit bicarbonate buffer containing 5% (w/v) bovine serum albumin, 40 mg/ml creatinine, 5 mM glucose, 3% mannitol, and 8 amino acids (methionine, 0.5 mM; alanine, 2 mM; glycine, 5 mM; serine, 2 mM; arginine, 1 mM; proline, 2 mM; isoleucine, 1 mM; and aspartic acid, 3 mM) (6), was aerated with 95% O₂ and 5% CO₂ and was kept at 37°C. Then, the kidney was perfused with Krebs-Henseleit bicarbonate buffer solution containing 10 µM vancomycin, p-aminohippurate, cimetidine and arbekacin, or vancomycin (10 µM) with 50 µM quinidine, fosfomycin and imipenem/cilastatin (in terms of imipenem content). After a short stabilizing period, three consecutive 5-minute clearance studies were performed. Urine samples were obtained over three 5-minute periods. The kidney was removed at the end of the experiment and blotted, weighed and homogenized in three volumes of saline for determination of vancomycin. All samples were stored at −20°C until analysis.

### Analytical Methods

The concentrations of creatinine, urea nitrogen and glucose were measured using the Jaffé method, urease-indophenol and o-toluidine, respectively, with kits obtained from Wako Pure Chemical Industries (Osaka, Japan). The sodium concentration was determined using an ion meter (Horiba F-8AT, Kyoto, Japan) with an ion-specific electrode (Horiba Sera-100, Kyoto, Japan).

The concentrations of vancomycin and cimetidine were determined by high-performance liquid chromatography (HPLC) as described previously (7). Briefly, the chromatograph (LC-10A, Shimadzu, Kyoto, Japan) was equipped with an SPD-10AV variable wavelength UV detector (Shimadzu) adjusted to 235 nm and an analytical pH reversed-phase column (Cosmosil 5Ph packed column, 15 cm × 4.6 mm, Naecali Tesque, Kyoto, Japan). The mobile phase consisted of 50 mM sodium phosphate buffer with 1 mM sodium lauryl sulfate (pH3.3)-acetonitrile, 79:21. The flow rate was 1.0 ml/min and the column temperature was maintained at 40°C. p-Aminomhippurate concentrations were also assayed by HPLC according to the method of Hori et al. (8). Arbekacin concentrations were determined by fluorescence polarization immunoassay with a TDx analyzer (Dainabot Laboratories, Tokyo, Japan).

Protein binding of vancomycin and cimetidine in the perfusate was determined by the ultrafiltration method using a micropartition system (MPS-1; Amicon, Beverly, MA, USA), and those of arbekacin and p-aminohippurate were similarly determined with Ultrafree®-MC (Millipore, Bedford, MA, USA). The unbound fraction of the drug was expressed as the ratio of its concentration in the ultrafiltrate to that in the perfusate.

### Data Analysis

Pharmacokinetic parameters of drugs in the perfused kidney were calculated based on standard procedures for each experimental period (7). That is, the renal clearance of the drugs was obtained as the urinary excretion rate divided by their concentrations in the perfusate. The renal clearance of unbound drugs was determined as the renal clearance over the unbound fraction. The excretion ratios of the drugs (ER) were estimated as the unbound renal clearance over the glomerular filtration rate (GFR; assumed equal to the renal clearance of creatinine). The reabsorption of glucose or sodium was defined as: (1 — renal clearance/GFR) × 100. In each experiment, the clearance of the drugs was estimated as the mean of three experimental periods.

### Statistical Analysis

Each experiment was performed with more than four rats. Data are expressed as means ± s.e.m. of separate experiments. Statistical significance of differences between mean values was calculated using the non-paired t-test. Multiple comparisons were performed by analysis of variance (ANOVA) followed by Scheffé's test for multiple comparisons provided that the variances of groups were similar. If this was not the case, a Scheffé-type test following Kruskal-Wallis analysis was applied. P values of less than 0.05 (two-tailed) were considered to be significant.

### RESULTS

The plasma concentrations of creatinine and urea nitrogen in rats were markedly increased two days after the injection of vancomycin (300 and 500 mg/kg), and dose-dependent increases in these parameters were also observed (Table I). With co-treatment with fosfomycin or imipenem/cilastatin, no marked elevation was observed in the plasma concentrations of creatinine or urea nitrogen, and the protective effects of the

### Table I. Effects of Fosfomycin and Imipenem/Cilastatin on Vancomycin-induced Nephrotoxicity

<table>
<thead>
<tr>
<th></th>
<th>Plasma Creatinine (mg/dl)</th>
<th>Plasma Urea Nitrogen (mg/dl)</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.46±0.02</td>
<td>13.7±1.2</td>
</tr>
<tr>
<td>+Fosfomycin (300 mg/kg)</td>
<td>0.62±0.03</td>
<td>13.3±1.2</td>
</tr>
<tr>
<td>+Imipenem/cilastatin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(150/150 mg/kg)</td>
<td>0.55±0.06</td>
<td>15.0±0.7</td>
</tr>
<tr>
<td>Vancomycin (300 mg/kg)</td>
<td>0.68±0.06</td>
<td>23.6±1.2</td>
</tr>
<tr>
<td>+Fosfomycin (300 mg/kg)</td>
<td>0.48±0.04</td>
<td>14.6±0.5</td>
</tr>
<tr>
<td>Vancomycin (500 mg/kg)</td>
<td>0.73±0.08</td>
<td>41.3±7.5</td>
</tr>
<tr>
<td>+Fosfomycin (300 mg/kg)</td>
<td>0.58±0.06</td>
<td>13.8±1.0</td>
</tr>
<tr>
<td>+Imipenem/cilastatin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(150/150 mg/kg)</td>
<td>0.62±0.06</td>
<td>13.8±1.4</td>
</tr>
</tbody>
</table>

* Plasma samples were collected two days after the intravenous injection of vancomycin. Each value represents the mean ± s.e.m. for 5 rats.

* P < 0.05, significantly different from the control group.

* P < 0.05, significantly different from vancomycin alone (300 and 500 mg/kg) groups, respectively.