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

Tsutomu Nakamura,[1] Yukiya Hashimoto,[1] Toshiyuki Kokuryo,[1] and Ken-Ichi Inui[1,2]

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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 cimetidine, 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 ototoxicity 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, cimetidine 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, cimetidine, 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

[1] Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan.

[2] To whom correspondence should be addressed. (e-mail: inui@kuhp.kyoto-u.ac.jp)

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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% O_2 + 5% CO_2 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, Nacalai 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-Aminohippurate 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[a]

	Plasma Creatinine (mg/dl)	Plasma Urea Nitrogen (mg/dl)
Control	0.46±0.02	13.7±1.2
+Fosfomycin (300 mg/kg)	0.62±0.03[b]	13.3±1.2
+Imipenem/cilastatin (150/150 mg/kg)	0.53±0.06	15.0±0.7
Vancomycin (300 mg/kg)	0.68±0.06	23.6±1.2
+Fosfomycin (300 mg/kg)	0.48±0.04[c]	14.6±0.5[c]
Vancomycin (500 mg/kg)	0.78±0.08	41.3±7.5
+Fosfomycin (300 mg/kg)	0.58±0.06	13.8±1.0[c]
+Imipenem/cilastatin (150/150 mg/kg)	0.62±0.06	13.8±1.4[c]

[a] Plasma samples were collected two days after the intravenous injection of vancomycin. Each value represents the mean ± s.e.m. for 5 rats.
[b] $P < 0.05$, significantly different from the control group.
[c] $P < 0.05$, significantly different from vancomycin alone (300 and 500 mg/kg) groups, respectively.

Table II. Renal Function and Pharmacokinetic Parameters of Vancomycin, p-Aminohippurate, Cimetidine and Arbekacin in the Perfused Rat Kidney[a]

Parameter	Vancomycin	p-Aminohippurate	Cimetidine	Arbekacin
Glucose reabsorption (%)	91.1±1.5	93.5±0.7	89.1±1.4	92.7±0.6
Sodium reabsorption (%)	69.7±3.5	88.0±2.1	65.8±3.6	84.0±1.5
Water reabsorption (%)	57.5±3.3	73.2±1.8	49.4±3.5	70.6±1.6
GFR (ml/min)	0.61±0.08	0.40±0.05	0.31±0.04	0.48±0.05
Urine flow rate (ml/min)	0.26±0.05	0.11±0.02	0.16±0.02	0.14±0.01
Unbound fraction	0.54±0.03	0.71±0.01	0.70±0.07	0.85±0.03

[a] The rat kidney was perfused with a solution containing vancomycin, p-aminohippurate, cimetidine or arbekacin (10 μM) at a constant flow rate of 16 ml/min. Each value represents the mean±s.e.m. for 4-5 rat kidneys.

antibiotic were more clearly seen in the concentration of urea nitrogen compared with creatinine.

The rat kidney was perfused at a constant flow rate of 16 ml/min. Under these conditions, the perfusion pressure was maintained at approximately 120 mmHg, and did not change significantly throughout the experiment. Table II shows the viability of the perfused kidney. The GFR, urine flow rate and the reabsorption of glucose, sodium and water were not changed throughout the experiment. The urinary excretion rates of vancomycin, p-aminohippurate (anionic drug), cimetidine (cationic drug) and arbekacin (aminoglycoside antibiotic) were also fairly constant during the three experimental periods. Fig. 1 shows the excretion ratios of vancomycin and various drugs in the perfused rat kidney. The ER value of vancomycin was 1.03 ± 0.05. On the other hand, those of p-aminohippurate, cimetidine and arbekacin were 7.09 ± 0.30, 4.49 ± 0.33 and 0.451 ± 0.043, respectively.

Table III summarizes the pharmacokinetic parameters of vancomycin (10 μM) with/without co-treatment with quinidine, fosfomycin, or imipenem/cilastatin (50 μM). None of these three drugs or vancomycin had any influence on the viability of the perfused kidney. In the kidney perfused with imipenem/cilastatin, the ER and renal clearance of unbound vancomycin were significantly decreased. The renal clearance was also decreased, although this was not significant. With co- perfusion of fosfomycin, there were no significant differences in the kinetic parameters of vancomycin, although the ER of vancomycin decreased similarly to that in the imipenem/cilastatin group (P = 0.0613). On the other hand, in the presence of quinidine, the ER of vancomycin increased significantly. The ratios of vancomycin concentration in the kidney to the unbound concentration in perfusate were also estimated in the absence and presence of these drugs. No significant differences were observed in the ratio among the groups: 1.39 ± 0.06 in the control and 1.34 ± 0.09, 1.33 ± 0.21 and 1.20 ± 0.10 in the quinidine, fosfomycin and imipenem/cilastatin co-perfused groups, respectively.

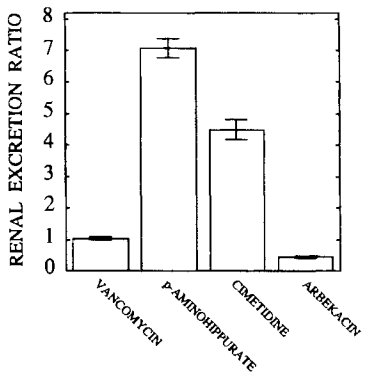

Fig. 1. Renal excretion ratios of various drugs (10 μM) in the perfused rat kidney.

DISCUSSION

Although vancomycin is widely used to treat infection with *methicillin-resistant staphylococci,* this drug is frequently associated with significant nephrotoxicity at high plasma concentrations (2). In the present study, induction of renal failure and elevation of plasma concentrations of creatinine and urea nitrogen were observed in rats administered vancomycin at doses of 300 and 500 mg/kg (Table I). Vancomycin is eliminated mainly by the kidney, and so it is important to understand its renal handling to determine the mechanism of its nephrotoxicity. We previously studied the renal handling of vancomycin in rats by *in vivo* clearance and reported that the antibiotic is secreted via renal tubules. Therefore, it is important to investigate the renal handling of vancomycin in the presence of the nephroprotective drugs fosfomycin and imipenem/cilastatin. To evaluate the renal handling of vancomycin in detail, the urinary excretion of vancomycin was investigated and compared with those of p-aminohippurate, cimetidine and arbekacin using the perfused rat kidney model. We further investigated the urinary excretion of vancomycin in the presence of quinidine, fosfomycin or imipenem/cilastatin. The control vancomycin concentration was designed to mimic that in our previous study, 10 μM, about 15 μg/ml.

The ER value of vancomycin was markedly less than those of p-aminohippurate and cimetidine, but larger than that of arbekacin (Fig. 1). p-Aminohippurate and cimetidine are typical substrates of organic anion and cation transporters, respectively, and are secreted via the renal proximal tubules (9,10). Their ER values (7.09 ± 0.30 and 4.49 ± 0.33, respectively), greater than unity, indicated that the renal tubular function, especially secretion, was viable in the perfused kidney. In our previous study, probenecid, a potent inhibitor of the renal organic anion transport system, did not affect the renal handling of vancomycin (4). Moreover, the renal secretion of vancomycin was less than that of p-aminohippurate in the present study. Therefore, vancomycin may not be transported by the organic anion transport system.

Table III. Effects of Various Drugs on the Renal Excretion of Vancomycin[a]

Parameter	Control	+Quinidine	+Fosfomycin	+Imipenem/cilastatin
GFR (ml/min)	0.526±0.061	0.322±0.018	0.402±0.082	0.398±0.076
Urine flow rate (ml/min)	0.233±0.038	0.168±0.014	0.131±0.027	0.132±0.039
Unbound fraction of vancomycin	0.518±0.017	0.529±0.014	0.519±0.035	0.635±0.049
Renal clearance (ml/min)	0.290±0.038	0.219±0.013	0.181±0.039	0.213±0.056
Renal clearance of unbound vancomycin	0.561±0.072	0.415±0.019	0.341±0.057	0.320±0.058[b]
Excretion ratio	1.07±0.04	1.29±0.02[b]	0.872±0.061	0.823±0.060[b]
Tissue/perfusate ratio of unbound vancomycin	1.39±0.06	1.34±0.09	1.33±0.21	1.20±0.10
n =	6	6	5	5

[a] The rat kidney was perfused with a solution containing vancomycin (10 μM) alone or with quinidine, fosfomycin (50 μM) or imipenem/cilastatin (50/42 μM) at a constant flow rate of 16 ml/min. Each value represents the mean±s.e.m.
[b] $P < 0.05$, significantly different from the control value.

We previously reported that quinidine, which can inhibit the renal organic cation transport system, decreases partially the total clearance of vancomycin (4). However, quinidine may have a hemodynamic effect and influence the pharmacokinetics of vancomycin. Therefore, it was unclear whether the secretion of vancomycin in rat renal tubules is mediated by the organic cation transport system. In this study, the effects of quinidine on the tubular secretion of vancomycin were examined in the perfused rat kidney under conditions in which circulatory effects of quinidine were eliminated. Here, the renal tubular secretion of vancomycin was markedly less than that of the cationic drug cimetidine (Fig. 1). The excretion ratio of vancomycin was not reduced in the presence of quinidine, and there was no significant difference in the tissue/perfusate ratio of vancomycin with or without quinidine (Table III). Therefore, the phenomenon observed *in vivo* may be explained not by the inhibition of secretion mediated by the organic cation transport system, but partly by a decrease in the effective renal plasma flow (4).

Arbekacin is an aminoglycoside antibiotic, and similarly to vancomycin this antibiotic is used to treat infections with *methicillin-resistant staphylococci* and is eliminated mainly by the kidney (11). The ER value of arbekacin was 0.451 ± 0.043, smaller than unity, indicating that arbekacin was reabsorbed via renal tubules in the perfused kidney (Fig. 1). This result was consistent with the previous report (12). On the other hand, the ER value of vancomycin was 1.03 ± 0.05, suggesting that vancomycin might be reabsorbed as well as secreted via renal tubules. Although vancomycin and aminoglycoside have frequently been reported to be associated with significant nephrotoxicity, the mechanisms of their toxic effects have not been clarified. It has been reported that the nephrotoxicity of aminoglycoside is induced by accumulation of the drug from the brush-border membrane in the renal proximal tubules (13,14), and the present results supported these reports (Fig. 1). Some investigators have suggested that the mechanism of vancomycin toxicity is similar to that of aminoglycoside with respect to accumulation in the kidney (15,16). We have already reported that tubular secretion is related to the renal excretion of vancomycin, and suggested that the mechanism of accumulation of vancomycin in the kidney is different from that of aminoglycoside due to the differences in their respective renal handling, i.e. secretion and reabsorption (4).

Fosfomycin and imipenem/cilastatin have been reported to have protective effects against the nephrotoxicity of vancomycin (3), and they has been evaluated for the purpose of clinical protection against the nephrotoxic effects of some drugs (17,18). In the present study, fosfomycin and imipenem/cilastatin suppressed the increases in plasma concentrations of creatinine and urea nitrogen due to the nephrotoxic effects of vancomycin (Table I). Thus, we investigated the renal handling of vancomycin in the presence of these nephroprotective drugs. In this study, the accumulation of vancomycin in the kidney was also evaluated as the tissue/perfusate ratio of unbound vancomycin in the presence of fosfomycin or imipenem/cilastatin. The accumulation of vancomycin was not significantly changed by co-treatment with fosfomycin or imipenem/cilastatin, although slight decreases were detected (Table III). On the other hand, fosfomycin and imipenem/cilastatin decreased the excretion ratio of vancomycin in the perfused kidney, which indicated that the drugs inhibited the tubular transport of vancomycin. Therefore, this inhibition in renal tubules may be at least a partially related to their protective effects against vancomycin nephrotoxicity. However, the tubular secretion of drugs consists of the transport process across both the basolateral and brush-border membranes of the renal tubular epithelium. It is not known on which membrane side fosfomycin and imipenem/cilastatin inhibited the transport of vancomycin. Accordingly, further studies are needed to understand the transport mechanisms of vancomycin in the renal epithelium and the protective effects of concomitantly administered drugs against its nephrotoxicity.

In conclusion, we investigated the effects of fosfomycin and imipenem/cilastatin on vancomycin-induced nephrotoxicity and on the renal excretion of vancomycin in rats. The results suggested that the protective effects of fosfomycin and imipenem/cilastatin against the nephrotoxicity of vancomycin might be partially due to reduction of the accumulation of vancomycin in renal tubules. These findings may provide new insight into the clinical use of fosfomycin and imipenem/cilastatin with vancomycin and the underlying mechanism of clearance and/or toxicity of vancomycin in the kidney.

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