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Renal Excretion of Vancomycin in Rats with Acute Renal Failure

TSUTOMU NAKAMURA, TOSHIYUKI KOKURYO, MIKIHISA TAKANO AND KEN-ICHI INUI

Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan

Abstract

We have investigated the renal excretion of vancomycin in rats with acute renal failure (ARF) induced by uranyl nitrate or cisplatin.

The renal clearance of the antibiotic after uranyl nitrate or cisplatin injection was separately evaluated by calculating the glomerular filtration rate (GFR) and secretory clearance. The reduced renal clearance of vancomycin in these ARF rats was a result of a decrease in both GFR and secretory clearance. The extents of the decreases in GFR and in secretory clearance were not, however, proportional, the extent of the decrease in secretory clearance being more pronounced.

These results suggest that the renal tubular secretion of vancomycin was reduced more predominantly than glomerular filtration in these ARF models.

Vancomycin hydrochloride, a glycopeptide antibiotic, is used for clinical treatment of infections with methicillin-resistant staphylococci (Cook & Farrar 1978). Because it can induce nephrotoxicity at high plasma concentrations (Cook & Farrar 1978), routine monitoring of the plasma concentration is recommended.

Because vancomycin is eliminated mainly by the kidney, renal failure has a large influence on rate of elimination (Lee et al 1956-1957). However, little is known about the renal excretion of vancomycin in patients with rapidly changing renal function (Leader et al 1995). We have previously studied the renal excretion of vancomycin by in-vivo clearance in normal rats, and showed that the antibiotic was secreted in the renal tubules (Nakamura et al 1996). It is, therefore, necessary to evaluate glomerular filtration rate and secretory clearance separately.

In this study the excretion of vancomycin in renal failure was investigated by studying in-vivo clearance by rats with acute renal failure induced by uranyl nitrate or cisplatin. The results suggested that renal tubular function was more predominantly impaired than glomerular filtration.

Materials and Methods

Materials

Vancomycin hydrochloride was provided by Shionogi (Osaka, Japan). Uranyl nitrate was obtained from Merck (Darmstadt, Germany). Cisplatin for injection was purchased from Bristol-Myers Squibb (Tokyo, Japan). Other chemicals were of the highest purity available.

Induction of acute renal failure

Acute renal failure (ARF) was induced in male Wistar albino rats, 200–250 g, by administration of either uranyl nitrate (10 mg kg^{-1}, s.c., Briplatin Injection (10 mg mL^{-1})) or with cisplatin (5 mg kg^{-1}, i.p., in normal saline (0·5 mg mL^{-1})). Normal healthy rats injected with saline served as the control group for each ARF group. Vancomycin clearance in rats with ARF was determined 1, 2 and 3 days after uranyl nitrate injection, and 2 and 3 days after cisplatin injection.

Correspondence: K. Inui, Department of Pharmacy, Kyoto University Hospital, Sakyo-ku, Kyoto 606-01, Japan.

In-vivo clearance study

Control and ARF rats were anaesthetized with pentobarbital (50 and 30 mg kg^{-1}, respectively, i.p.). Catheters were inserted into the left femoral artery and the right femoral vein for blood sampling and drug infusion, respectively. Urine was collected from the urinary bladder catheterized after a suprapubic incision. The loading dose of vancomycin (0·5 mg) required to give a plasma concentration of drug equal to that at steady-state was administered with inulin (10 mg) and mannitol (40 mg) through the femoral vein. The loading dose remaining in the catheter was forced in with saline. Thereafter vancomycin was continuously infused (0·5 mg h^{-1}) concomitantly with inulin (20 mg h^{-1}) and mannitol (100 mg h^{-1}) at 2·2 mL h^{-1} over a period of 1 h for equilibration. Mannitol was administered to maintain a sufficient and constant urine flow rate. After 1-h infusion for equilibration, three consecutive 20-min clearance studies were performed. All blood samples for the antibiotic and inulin assays were collected at the midpoint of each experimental period, and centrifuged for plasma sampling. Urine samples obtained during the three periods were diluted 1 : 100 with saline. Plasma and urine samples were stored at −20°C until analysis. Animal experiments were performed in accordance with the Guideline for Animal Experiments of Kyoto University.

Analytical methods

Samples were assayed within 3 days of collection. The concentrations of vancomycin in the plasma and urine were determined by high-performance liquid chromatography (HPLC). The chromatograph, LC-10A (Shimadzu, Kyoto, Japan), was equipped with an SPD-10AV variable wavelength UV detector (Shimadzu) adjusted at 235 nm and an analytical C_{18} reversed-phase column (Chemcosorb 5-ODS-H, 15 cm × 4·6 mm, Chemco, Osaka, Japan). The mobile phase was 0·05 M sodium phosphate buffer (pH 5·5)-acetonitrile, 93 : 7. The flow rate was 1·0 mL min^{-1} and the column tem-

perature was maintained at 40°C. The concentration of vancomycin was calculated from the peak height by use of a calibration curve. Samples were deproteinized and filtered as described elsewhere (Nakamura et al 1996) and 50 μL of the filtrate was injected into the column for vancomycin assay. Inulin concentrations in plasma and urine were analysed spectrophotometrically by a modification of the method of Dische & Borenfreund (1951). The concentrations of urea nitrogen and creatinine in plasma and of glucose in urine were measured using urease-indophenol, the Jaffé method and *o*-toluidine, respectively, with kits obtained from Wako Pure Chemical Industries (Osaka, Japan). The protein-binding of vancomycin was determined by ultrafiltering a plasma sample with a micropartition system (MPS-1; Amicon, Beverly, MA, USA). The free fraction of vancomycin was expressed as the ratio of the vancomycin concentration in the ultrafiltrate to that in plasma.

Data analysis

Pharmacokinetic parameters were calculated by use of standard procedures for each experimental period. The total body vancomycin clearance (CL_t) was calculated as the infusion rate divided by the steady-state plasma concentration (C_{pss}). The renal clearance (CL_r) was obtained from the urinary excretion rate divided by C_{pss}. The renal clearance of unbound vancomycin ($CL_{r,f}$) was determined by dividing CL_r by the free fraction. The excretion ratio of vancomycin (ER) was estimated as $CL_{r,f}$ divided by the glomerular filtration rate (GFR; assumed equal to the CL_r of inulin). The net renal secretory clearance of unbound vancomycin (CL_s) was calculated by subtracting GFR from $CL_{r,f}$. In each experiment, the clearance of vancomycin and inulin was estimated as the mean of the results from three experimental periods.

Statistical analysis

Each experiment was performed on groups of at least four rats. Data are expressed as the means ± s.e.m. of separate experiments. Statistical comparisons were completed by the appropriate analysis of variance model; Dunnett's test for multiple comparisons was used to show if the variances of groups were similar. If not, a Dunn-type test was applied after Kruskal–Wallis analysis. *P* values of less than 0·05 (two-tailed) were considered significantly different.

Results

Renal excretion of vancomycin by ARF rats

In all experiments, we confirmed that the plasma concentration of vancomycin was in a steady state during the three experimental periods in control and ARF rats. The concentrations of creatinine and urea nitrogen in the plasma and of glucose in urine were markedly increased in all animals from 2 days after administration of uranyl nitrate (Table 1) and cisplatin (Table 2).

Table 3 shows the pharmacokinetic parameters of vancomycin in rats with uranyl nitrate-induced ARF. The plasma concentrations of vancomycin in ARF rats were significantly higher than those in control animals, and reached 2·5-fold increases 3 days after uranyl nitrate injection. With the progress of renal failure, both the total and renal clearance of the drug decreased significantly. Although $CL_{r,f}$, ER and CL_s also decreased significantly, the free fraction of vancomycin was not altered in days 1–3 in rats with ARF. The extents of the decrease in the ER were similar on days 2 and 3.

Table 1. Effect of uranyl nitrate on creatinine and urea nitrogen in the plasma and on glucose in the urine.

	Plasma creatinine (mg/100 mL)	Urea nitrogen (mg/100 mL)	Urine glucose (mg/100 mL)
Control	0·56 ± 0·1	13·0 ± 0·7	30·5 ± 5·0
Day 1	0·73 ± 0·4	23·2 ± 11·6	16·8 ± 6·3
Day 2	1·10 ± 0·2	29·0 ± 1·2*	747·4 ± 9·3*
Day 3	2·59 ± 0·4	60·4 ± 13·1*	587·8 ± 29·1

Rats were injected with uranyl nitrate (10 mg kg^{-1}, s.c.). After 1 to 3 days plasma and urine were collected for measurements. Each value represents the mean ± s.e.m. of results from four to seven rats. *$P < 0·05$, significantly different from the control value.

Table 2. Effect of cisplatin on creatinine and urea nitrogen in the plasma and on glucose in the urine.

	Plasma creatinine (mg/100 mL)	Urea nitrogen (mg/100 mL)	Urine glucose (mg/100 mL)
Control	0·28 ± 0·1	15·1 ± 1·9	42·1 ± 9·5
Day 2	1·05 ± 0·4	35·4 ± 11·6*	129·7 ± 29·6*
Day 3	1·66 ± 0·3*	35·4 ± 6·6*	407·6 ± 163·2*

Rats were injected with cisplatin (5 mg kg^{-1}, i.p.). After 2 or 3 days plasma and urine were collected for measurements. Each value represents the mean ± s.e.m. of results from four to five rats. *$P < 0·05$, significantly different from the control value.

Table 4 shows the pharmacokinetic parameters of vancomycin in rats given cisplatin. The changes in the parameters in rats with ARF induced with cisplatin were similar to those in rats with ARF induced with uranyl nitrate. In rats with cisplatin-induced ARF, C_{pss} was significantly higher than in control rats, and CL_t, CL_r, $CL_{r,f}$, GFR, ER and CL_s of vancomycin decreased significantly.

Fig. 1 shows the relationship between GFR and the secretory clearance of vancomycin in rats with ARF induced by uranyl nitrate or cisplatin. On 1, 2 and 3 days after uranyl nitrate injection, the values of GFR were 62, 36 and 14% of the control value, whereas those of CL_s of vancomycin were 39, 9 and 4%, respectively. In cisplatin-treated rats, GFR values 2 and 3 days after treatment were 74 and 37% of the control value; on the same days CL_s of vancomycin was 41 and 7%, respectively.

Discussion

Vancomycin is frequently given to patients with acute renal failure, but the fundamental pharmacokinetics of the antibiotic under these conditions have not been fully elucidated. Our studies suggested that vancomycin is secreted in the renal tubules of rats (Nakamura et al 1996). In this report, we separately evaluated glomerular filtration and renal tubular secretion of vancomycin to understand in more detail the renal handling of vancomycin in rats with acute renal failure induced by uranyl nitrate or cisplatin.

Uranyl nitrate injection is an established nephrotoxic model that selectively causes renal dysfunction (Flamenbaum et al

Table 3. Effect of uranyl nitrate on the urinary excretion of vancomycin.

	Control (n = 8)	Day 1 (n = 5)	Day 2 (n = 5)	Day 3 (n = 5)
Steady-state plasma concentration (μg mL^{-1})	5·47 ± 0·27	6·84 ± 0·34*	10·3 ± 0·44*	13·7 ± 0·42*
Total body clearance (mL min^{-1})	1·71 ± 0·09	1·36 ± 0·07*	0·89 ± 0·04*	0·67 ± 0·02*
Renal clearance (mL min^{-1})	1·46 ± 0·07	0·86 ± 0·05*	0·41 ± 0·05*	0·17 ± 0·05*
Renal clearance of unbound vancomycin (mL min^{-1})	4·29 ± 0·25	2·21 ± 0·13*	1·02 ± 0·13*	0·40 ± 0·10*
Glomerular filtration rate (mL min^{-1})	2·30 ± 0·15	1·42 ± 0·08*	0·83 ± 0·06*	0·33 ± 0·10*
Excretion ratio	1·88 ± 0·08	1·56 ± 0·08*	1·22 ± 0·12*	1·27 ± 0·06*
Net renal secretory clearance of unbound vancomycin (mL min^{-1})	1·99 ± 0·16	0·78 ± 0·10	0·19 ± 0·10*	0·07 ± 0·01*

Vancomycin was infused intravenously at 2·0 mg h^{-1} 1, 2 or 3 days after injection of uranyl nitrate. Each value represents the mean ± s.e.m. *$P < 0·05$, significantly different from the control value.

Table 4. Effect of cisplatin on the urinary excretion of vancomycin.

	Control (n = 4)	Day 2 (n = 5)	Day 3 (n = 4)
Steady-state plasma concentration (μg mL^{-1})	5·82 ± 0·57	9·18 ± 0·91*	12·8 ± 0·63*
Total body clearance (mL min^{-1})	1·62 ± 0·15	1·05 ± 0·12*	0·72 ± 0·03*
Renal clearance (mL min^{-1})	1·30 ± 0·10	0·72 ± 0·08*	0·33 ± 0·04*
Renal clearance of unbound vancomycin (mL min^{-1})	3·25 ± 0·20	1·92 ± 0·29*	0·75 ± 0·11*
Glomerular filtration rate (mL min^{-1})	1·75 ± 0·11	1·30 ± 0·15*	0·64 ± 0·07*
Excretion ratio	1·86 ± 0·08	1·47 ± 0·16*	1·15 ± 0·07*
Net renal secretory clearance of unbound vancomycin (mL min^{-1})	1·50 ± 0·14	0·62 ± 0·21*	0·10 ± 0·05*

Vancomycin was infused intravenously at 2·0 mg h^{-1} 2 or 3 days after injection of cisplatin. Each value represents the mean ± s.e.m. *$P < 0·05$, significantly different from the control value.

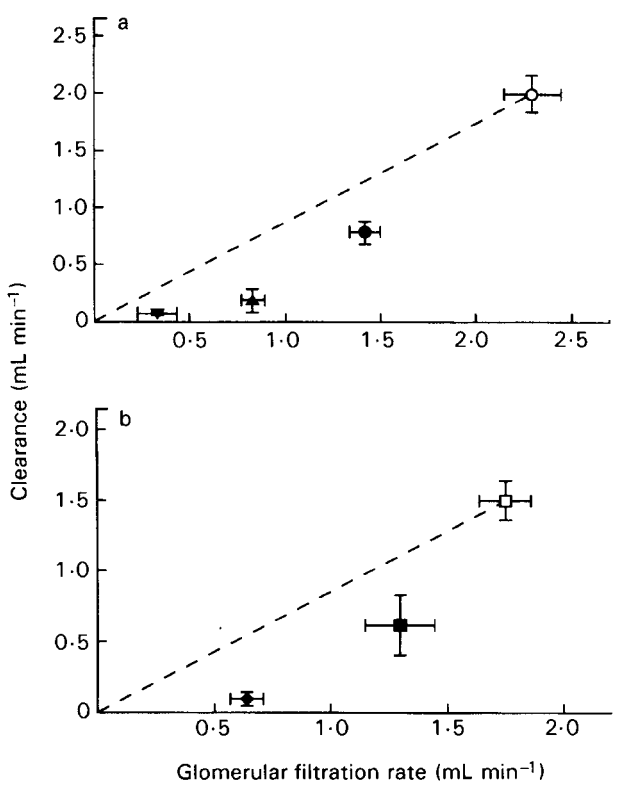

FIG. 1. Relationship between glomerular filtration rate and CL_s for vancomycin in rats with acute renal failure induced by a, uranyl nitrate or b, cisplatin. a, Control (○) and uranyl nitrate-treated rats were examined one (●), two (▲) or three (▼) days after the injection. b, Control (□) and cisplatin-treated rats were examined 2 (■) or 3 (◆) days after the injection.

1974). In rats with acute renal failure induced by uranyl nitrate, the concentration of creatinine in the plasma and of glucose in the urine increased, confirming that glomerular filtration is impaired and that renal tubular function such as re-absorption is decreased (Table 1). The renal secretion of model drugs, such as tetraethylammonium and *p*-aminohippurate, is decreased in-vivo in rats with acute renal failure induced by uranyl nitrate (Lin & Lin 1988). We also investigated the transport of tetraethylammonium and *p*-aminohippurate in ARF rats using the renal brush-border, basolateral membrane vesicles, and the perfused kidney isolated from rats treated with uranyl nitrate. Tetraethylammonium transport was reduced, whereas that of *p*-aminohippurate was not altered in the brush-border membrane vesicles (Hori et al 1985a), and the opposite was true in basolateral membrane vesicles isolated from the kidneys of ARF rats (Inui et al 1989). A similar phenomenon has been identified in a moderate acute renal failure model using the perfused rat kidney (Tanigawara et al 1990). We suggested that vancomycin is partly secreted by an organic cation transport system in renal tubules, the same as that by which tetraethylammonium is transported (Nakamura et al 1996). The decrease in the CL_s of vancomycin in ARF rats might, therefore, be a result of a dysfunctional secretory system in renal tubules.

Cisplatin impairs the kidney, particularly the renal proximal tubules (Dobyan et al 1980; Jones et al 1985). In this study, the CL_s of vancomycin was decreased in cisplatin-induced ARF rats as in the uranyl nitrate-induced model, suggesting that the reduced renal secretion of vancomycin is due to a transport dysfunction in the renal tubules.

Because glomerular filtration and tubular secretion are important processes for the renal excretion of vancomycin,

they were separately evaluated using the values of GFR and the CL_s of the antibiotic in ARF rats. Both GFR and the CL_s were decreased, but not to the same extent in ARF induced by uranyl nitrate and cisplatin. The decrease in the CL_s was more pronounced than that in GFR, especially 2 days after uranyl nitrate and 3 days after cisplatin administration. This phenomenon is also associated with some other drugs (Hori et al 1983; Maiza & Daley-Yates 1991) such as cimetidine, which is secreted in renal proximal tubules. The relationship between the changes in GFR and the CL_s of cimetidine was curvilinear in cisplatin-treated rats, as those changes were associated with vancomycin excretion in the present study.

In general, renal function is evaluated on the basis of GFR and the renal clearance of a drug is also estimated by use of the GFR. If in renal failure renal tubules are predominantly impaired as compared with the glomerulus, an unpredictable reduction in the renal excretion of the drug might, therefore, cause a remarkable increase in its plasma concentration, causing side effects. In contrast, in renal diseases such as glomerulonephritis, in which the glomerulus would be more severely impaired than the renal tubules, the secretory clearance would not decrease as in GFR, and the plasma concentration of the drug might not achieve the effective level (Maiza & Daley-Yates 1991). It is, therefore, necessary to understand not only changes in glomerular filtration but also those in the tubular secretion of a drug in renal failure. The use of phenolsulphonephthalein and *N*-1-methylnicotinamide have been proposed for the evaluation of these two functions simultaneously (Hori et al 1985b; Maiza & Daley-Yates 1993). Both phenolsulphonephthalein and *N*-1-methylnicotinamide are eliminated by glomerular filtration and tubular secretion in the kidney. These compounds have, furthermore, been used for adequate dose adjustment of drugs that are filtered by the glomeruli and secreted or reabsorbed in the renal tubules, or both. Thus, the renal handling of vancomycin in renal failure might be correctly evaluated by use of phenolsulphonephthalein or *N*-1-methylnicotinamide.

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