J. Pharm. Pharmacol. 1996, 48: 1197–1200
Received December 28, 1995
Accepted March 25, 1996

In-vivo Clearance Study of Vancomycin in Rats

TSUTOMU NAKAMURA, MIKIHISA TAKANO, MASATO YASUHARA* AND KEN-ICHI INUI

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

Abstract

The renal handling of vancomycin in rats and the effects of various drugs (probenecid, cimetidine and quinidine) on urinary excretion of the antibiotic were investigated by in-vivo clearance.

The vancomycin-to-inulin excretion ratio (ER) was greater than unity at various infusion rates of vancomycin. Quinidine, co-administered with vancomycin, significantly decreased the total, renal and net secretory clearance of the antibiotic. Cimetidine also decreased, though not significantly, the secretory clearance of vancomycin by about 20%, but probenecid did not.

These results suggested that vancomycin is secreted in renal tubules in rats, and that quinidine decreases the total clearance of vancomycin partly by inhibiting its tubular secretion in the kidney.

Vancomycin hydrochloride, a glycopeptide antibiotic, is frequently used to treat infections with methicillin-resistant staphylococci (Cook & Farrar 1978). Vancomycin however, may be ototoxic and nephrotoxic at high plasma concentrations (Cook & Farrar 1978). Therefore, to use vancomycin safely and effectively, monitoring of the drug concentration in plasma is often recommended for the appropriate adjustment of dose, dosage interval, or both.

Vancomycin is eliminated mainly by the kidney (Lee et al 1956-1957). However, the renal handling of vancomycin is still controversial and is not fully understood. Some investigators suggest that vancomycin is excreted by glomerular filtration, whereas others support the involvement of renal tubular secretion (Nielsen et al 1975; Nivoche et al 1982; Golper et al 1988; Rodvold et al 1988; Rybak et al 1990). In addition, there is little information concerning the mechanisms underlying tubular secretion of vancomycin in the kidney, if it occurs.

In the present study, we first examined the renal handling of vancomycin, specifically its tubular secretion, by in-vivo clearance technique in rats. The results suggested that vancomycin was secreted in renal tubules. We next studied the effects of various ionic drugs (probenecid, cimetidine and quinidine) on the renal handling of vancomycin to clarify the mechanism of its tubular secretion. The renal secretion of vancomycin was inhibited by quinidine, suggesting the possible involvement of the organic cation transport system.

Materials and Methods

Materials

Vancomycin hydrochloride was provided by Shionogi (Osaka, Japan). Probenecid was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Quinidine sulphate, cimetidine and inulin were purchased from Nacalai Tesque (Kyoto, Japan). All other chemicals were of the highest purity available.

*Present address: Department of Hospital Pharmacy, School of Medicine, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan.

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

In-vivo clearance study

The investigations were performed on male Wistar albino rats (200–250 g) by in-vivo clearance (Kamiya et al 1983; Kikkoji et al 1988). The animals were anaesthetized with pentobarbital administered by an intraperitoneal injection (50 mg kg^{-1}). When necessary, additional pentobarbital was administered to keep the animals anaesthetized throughout the experiments. Two catheters were inserted into the left femoral artery and the right femoral vein for blood sampling and for infusing drugs, respectively. Urine was collected from urinary bladder catheterized after a suprapubic incision. Vancomycin was administered alone or with probenecid, cimetidine or quinidine. The loading dose of vancomycin (0·1–4·0 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, a continuous infusion of vancomycin (0·1–4·0 mg h^{-1}) was started 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. Vancomycin was co-administered as follows. The dosages of probenecid (2·6 mg) and cimetidine (4·8 mg) were co-injected intravenously with the loading dose of vancomycin (2·0 mg). The loading dose of quinidine was omitted because of its acute circulatory effect. Thereafter, probenecid (9·7 mg h^{-1}), cimetidine (48 mg h^{-1}) or quinidine (8·5 mg h^{-1}) was infused simultaneously with vancomycin (2·0 mg h^{-1}) at 2·2 mL h^{-1}. After a 1-h infusion for equilibration, three consecutive 20-min clearance studies (periods 1–3) 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. After the experiments, the rats were killed under anaesthesia to obtain plasma samples. Plasma and urine samples were stored at −20°C until analysis.

Analytical methods

Samples were assayed within 3 days from the time of collection. The concentrations of vancomycin in plasma and urine

were determined by high-performance liquid chromatography (HPLC). A high-performance liquid chromatograph LC-3A (Shimadzu, Kyoto, Japan) was equipped with a variable wavelength UV detector SPD-6A (Shimadzu) adjusted at 235 nm and an analytical C_{18} reverse-phase column (Chemcosorb 5-ODS-H, 15 cm × 4·6 mm, Chemco Co., Osaka, Japan). The mobile phase consisted of 0·05 M sodium phosphate buffer (pH 5·5): acetonitrile = 93:7. The flow rate was 1·0 mL min^{-1} and the column temperature was maintained at 40°C. The concentration of vancomycin was calculated by measurement of peak height using a calibration curve. Samples (0·2 mL) were deproteinized by adding 0·3 mL of an acetone-10% trichloroacetic acid (1:2) mixture. The mixture was vortex mixed and centrifuged for 2 min at 13 000 rev min^{-1} in an Eppendorf centrifuge. To a 0·25-mL volume of the supernatant, 0·25 mL of 0·05 M phosphate buffer (pH 3·0) and 1·0 mL of ether were added. The mixture was vortex mixed for 10 s and then centrifuged for 1 min. The upper ether layer was aspirated and discarded. After remaining ether was removed, the water layer was passed through a 0·45-μm filter and 50 μL of the filtrate was injected into the column to assay vancomycin. Inulin concentrations in plasma and urine were analyzed by spectrophotometric assay with a modification of the method of Dische & Borenfreund (1951). The protein binding of vancomycin was determined by ultrafiltration of a plasma sample with a micropartition system (MPS-1; Amicon Corp., Beverly, MA, USA). The free fraction (f_u) of vancomycin was expressed as the ratio of the vancomycin concentration in the ultrafiltrate to that in plasma.

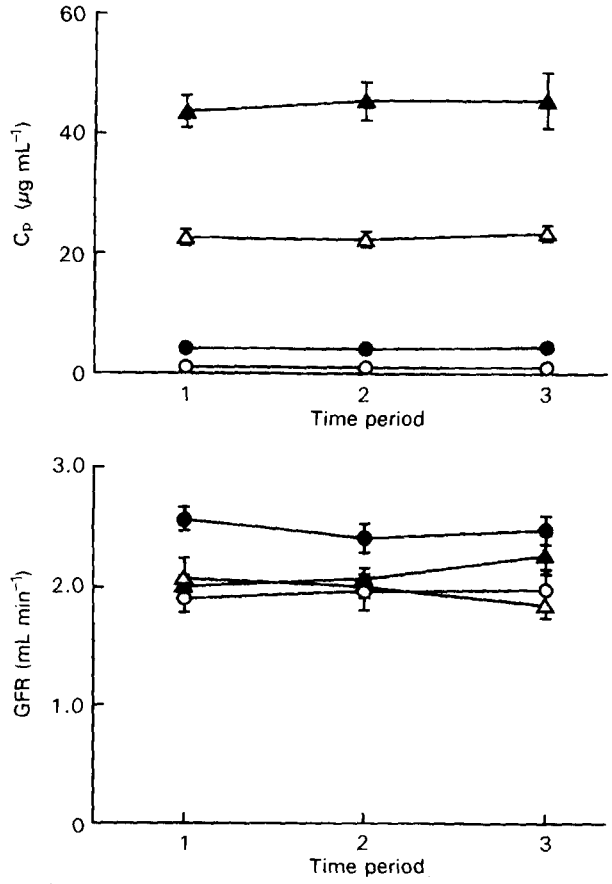

FIG. 1. Plasma concentration (C_p) of vancomycin and glomerular filtration rate (GFR) during the experimental periods. The plasma and urine samples were collected as described in the Materials and Methods. Infusion rate of vancomycin was 0.1 (○), 0.5 (●), 2.0 (△) or 4.0 (▲) mg h^{-1}. Period 1: 60–80 min; period 2: 80–100 min; period 3: 100–120 min after starting vancomycin infusion.

Data analysis

Pharmacokinetic parameters were calculated based on 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 as the urinary excretion rate (UV) divided by the C_{pss}. The renal clearance of unbound vancomycin ($CL_{r,f}$) were determined by dividing CL_r by f_u. The excretion ratio of vancomycin-to-inulin (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 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 comparisons were completed by the appropriate analysis of variance model and Scheffé's test for multiple comparisons provided that the variances of groups were similar. If not, a Scheffé-type test following Kruskal-Wallis analysis was applied. P values of less than 0·05 (two-tailed) were considered to be significantly different.

Results

As shown in Fig. 1, we confirmed that the plasma concentration of vancomycin was in a steady state and that glomerular filtration rate (GFR) was fairly constant during the three experimental periods. Table 1 shows the pharmacokinetic parameters obtained from the clearance studies at various infusion rates of vancomycin (0·1–4·0 mg h^{-1}). C_{pss} increased proportionally with increasing infusion rates and therefore the total clearance of vancomycin remained fairly constant over these rates. CL_r and $CL_{r,f}$ were not changed significantly in all experimental groups and the CL_r was more than 90% of the CL_t of vancomycin. CL_s, the calculated net renal secretory clearance, was 1·2–1·3 mL min^{-1}.

Table 2 summarizes the effect of various drugs on the renal handling of vancomycin at an infusion rate of 2·0 mg h^{-1}. In these experiments, the test compounds were probenecid (an anionic drug), cimetidine and quinidine (cationic drugs). In the group co-administered probenecid, there were no significant differences in the kinetic parameters of vancomycin compared with those in the control. When cimetidine was co-administered, there was no significant difference, although C_{pss} tended to increase and CL_t, CL_r, $CL_{r,f}$ and CL_s tended to decrease. On the other hand, in the presence of quinidine, C_{pss} increased and CL_t, CL_r, $CL_{r,f}$, GFR and CL_s decreased significantly. ER also decreased, though not significantly.

Table 1. Urinary excretion of vancomycin during the constant intravenous infusion of vancomycin.

Infusion rate (mg h^{-1})	C_{pss} ($\mu g\ mL^{-1}$)	UV ($\mu g\ min^{-1}$)	CL_t (mL min^{-1})	CL_r (mL min^{-1})	$CL_{r,f}$ (mL min^{-1})	GFR (mL min^{-1})	ER	CL_s (mL min^{-1})
0·1	1·18 ± 0·11	1·58 ± 0·18	1·54 ± 0·14	1·33 ± 0·06	3·25 ± 0·33	1·95 ± 0·14	1·67 ± 0·12	1·33 ± 0·29
0·5	4·26 ± 0·20	8·33 ± 0·39	2·09 ± 0·09	1·98 ± 0·11	3·70 ± 0·23	2·51 ± 0·10	1·47 ± 0·06	1·19 ± 0·16
2·0	22·8 ± 1·26	31·4 ± 1·96	1·57 ± 0·10	1·39 ± 0·07	3·24 ± 0·16	1·98 ± 0·09	1·65 ± 0·08	1·26 ± 0·13
4·0	44·0 ± 3·08	68·2 ± 3·48	1·62 ± 0·11	1·56 ± 0·05	3·25 ± 0·10	2·10 ± 0·09	1·56 ± 0·04	1·16 ± 0·06

Each value represents the mean ± s.e.m.; n = 5, 7, 8 and 5 for vancomycin infusion rates of 0·1, 0·5, 2·0 and 4·0 mg h^{-1}, respectively.

Table 2. Effect of the co-administration of various drugs on the urinary excretion of vancomycin.

	C_{pss} ($\mu g\ mL^{-1}$)	UV ($\mu g\ min^{-1}$)	CL_t (mL min^{-1})	CL_r (mL min^{-1})	$CL_{r,f}$ (mL min^{-1})	GFR (mL min^{-1})	ER	CL_s (mL min^{-1})
Control	21·9 ± 1·35	31·5 ± 2·84	1·63 ± 0·10	1·45 ± 0·14	3·12 ± 0·29	1·91 ± 0·14	1·62 ± 0·04	1·21 ± 0·16
Probenecid	21·8 ± 1·61	27·9 ± 1·97	1·64 ± 0·12	1·30 ± 0·13	3·14 ± 0·46	1·69 ± 0·15	1·84 ± 0·16	1·45 ± 0·35
Cimetidine	26·1 ± 1·57	30·3 ± 2·48	1·36 ± 0·08	1·19 ± 0·16	2·72 ± 0·32	1·77 ± 0·25	1·56 ± 0·05	0·96 ± 0·09
Quinidine	34·1 ± 3·80*	22·5 ± 2·46	1·06 ± 0·10*	0·71 ± 0·13*	1·41 ± 0·29*	1·04 ± 0·19*	1·34 ± 0·05	0·37 ± 0·11*

Vancomycin was infused intravenously at 2·0 mg h^{-1} without or with probenecid (9·7 mg h^{-1}), cimetidine (48 mg h^{-1}) and quinidine (8·5 mg h^{-1}). Each value represents the mean ± s.e.m.; n = 6, 5, 4, and 4 for control, probenecid, cimetidine, and quinidine groups, respectively. *$P < 0.05$, significantly different from the control value.

Discussion

Although vancomycin is widely used to treat infection with methicillin-resistant staphylococci, its fundamental pharmacokinetics have not been fully elucidated. This report describes the in-vivo effects of the changes in infusion rates and of various drugs on the urinary excretion of vancomycin using a rat model. The control of the latter studies was designed to mimic the clinical situation by setting the steady-state plasma concentration of vancomycin to about 20 $\mu g\ mL^{-1}$.

Net tubular secretion of vancomycin in rabbits (Nivoche et al 1982) and in humans (Rodvold et al 1988; Rybak et al 1990) has been suggested. On the other hand, there are some reports indicating that vancomycin is renally excreted via glomerular filtration (Nielsen et al 1975; Golper et al 1988). We calculated the excretion ratio of vancomycin in rats using the plasma concentration of free drug, and found that the excretion ratio of vancomycin was greater than unity in all infusion rates examined, indicating that this antibiotic is secreted by the renal tubules in rats. The ER values obtained were 1·5–1·7, suggesting that about 30–40% of vancomycin excreted in the urine is due to tubular secretion. Our results also showed that changes in the infusion rates had no effect on the net secretory clearance of vancomycin. The value of CL_s was nearly constant over a wide range of plasma vancomycin concentrations, suggesting that the putative vancomycin transport system may not be saturated under these experimental conditions.

The renal secretion of vancomycin implies transport across both the basolateral and brush-border membranes of renal tubular epithelium. In the kidney, many ionic drugs are excreted either by an organic anion transport system or by an organic cation transport system in proximal tubular cells (Pritchard & Miller 1993). Vancomycin has both carboxyl and nitrogen groups in its chemical structure. Therefore, it is interesting to test the effect of anionic (probenecid) and cationic (cimetidine, quinidine) drugs on the renal handling of vancomycin.

First, the effect of probenecid, a potent inhibitor of the renal organic anion transport system, was examined. However, probenecid did not affect the renal handling of vancomycin. Therefore, the organic anion transport system may not be involved in the renal secretion of vancomycin. We then studied the effects of cimetidine and quinidine, which are organic cations that can inhibit the renal organic cation transport system (Prichard & Miller 1993; Katsura et al 1993; Gross & Somogyi 1994). Quinidine significantly increased the C_{pss} of vancomycin and decreased the CL_t, CL_r, $CL_{r,f}$ and CL_s. ER was also decreased, though not significantly, because $CL_{r,f}$ as well as GFR decreased. The decreased renal clearance of vancomycin (CL_r, $CL_{r,f}$) should be partly due to the decrease in GFR. However, the decrease in CL_s cannot be explained by the decrease in GFR and it is likely that quinidine inhibited the renal secretion of vancomycin. Cimetidine showed similar, but weaker effects on the renal handling of vancomycin when compared with those by quinidine, and the changes in kinetic parameters were not significantly different.

Sokol (1991) has studied the transport mechanism of vancomycin by using rabbit renal membrane vesicles. Though he did not measure the transport of vancomycin itself, he showed that it inhibited the uptake of tetraethylammonium by basolateral membrane vesicles and that when preloaded in the vesicles, vancomycin stimulated the uptake of tetraethylammonium (trans-stimulation). Based on these results, the author concluded that vancomycin is transported by the organic cation transport system in rabbit renal basolateral membrane. These findings and ours suggest that the secretion of vancomycin in rat renal tubules is mediated by the organic cation transport system.

However, it is reported that both quinidine and cimetidine are also substrates for P-glycoprotein (Tsuruo et al 1984; Pan et al 1994). P-Glycoprotein is a membrane glycoprotein encoded by the multidrug resistant gene, and is expressed not only in multidrug-resistant tumour cells but also in normal tissues including the kidney (Gottesman & Pastan 1993). In the kidney, this glycoprotein is expressed in the brush-border membrane of proximal tubular cells, and functions as a drug-efflux pump with broad substrate specificity. In addition, the substrate specificity of P-glycoprotein partly overlaps that of the renal organic cation transport system (Dutt et al 1994). Therefore, the inhibitory effect of quinidine and cimetidine on the renal secretion of vancomycin should be carefully interpreted. Further studies are needed to clarify the mechanisms involved in the tubular secretion of vancomycin in the kidney.

In summary, vancomycin is secreted in the rat kidney and the net renal secretory clearance is fairly constant over a wide range of the plasma concentrations of vancomycin. Furthermore, quinidine may inhibit the renal secretion of vancomycin, resulting in decreased total clearance and increased plasma concentration of the antibiotic. The present findings provide useful information for further studies of the mechanisms underlying the renal transport of vancomycin.

References

Cook, F. V., Farrar, W. E. (1978) Vancomycin revisited. Ann. Intern. Med. 88: 813–818

Dische, Z., Borenfreund, E. (1951) A new spectrophotometric method for the detection and determination of keto sugars and trioses. J. Biol. Chem. 192: 583–587

Dutt, A., Heath, L. A., Nelson, J. A. (1994) P-glycoprotein and organic cation secretion by the mammalian kidney. J. Pharmacol. Exp. Ther. 269: 1254–1260

Golper, T. A., Noonan, H. M., Elzinga, L., Gilbert, D., Brummett, R., Anderson, J. L., Bennett, W. M. (1988) Vancomycin pharmacokinetics, renal handling, and nonrenal clearances in normal human subjects. Clin. Pharmacol. Ther. 43: 565–570

Gottesman, M. M., Pastan, I. (1993) Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu. Rev. Biochem. 62: 385–427

Gross, A. S., Somogyi, A. A. (1994) Interaction of the stereoisomers of basic drugs with the uptake of tetraethylammonium by rat renal brush-border membrane vesicles. J. Pharmacol. Exp. Ther. 268: 1073–1080

Kamiya, A., Okumura, K., Hori, R. (1983) Quantitative investigation of renal handling of drugs in rabbits, dogs, and humans. J. Pharm. Sci. 72: 440–443

Katsura, T., Takano, M., Tomita, Y., Yasuhara, M., Inui, K., Hori, R. (1993) Characteristics of organic cation transporter in rat renal basolateral membrane. Biochim. Biophys. Acta 1146: 197–202

Kikkoji, T., Kamiya, A., Inui, K., Hori, R. (1988) Urinary excretion and diuretic action of furosemide in rats: increased response to the urinary excretion rate of furosemide in rats with acute renal failure. Pharm. Res. 5: 699–703

Lee, C.-C., Anderson, R. C., Chen, K. K. (1956-1957) Vancomycin, a new antibiotic V. Distribution, excretion, and renal clearance. In: Welch, H., Marti-Ibanez, F. (eds) Antibiotics Annual. Medical Encyclopedia, New York, pp 82-89

Nielsen, H. E., Hansen, H. E., Korsager, B., Skov, P. E. (1975) Renal excretion of vancomycin in kidney disease. Acta Med. Scand. 197: 261–264

Nivoche, Y., Contrepois, A., Cremieux, A.-C., Carbon, C. (1982) Vancomycin in rabbits: pharmacokinetics, extravascular diffusion, renal excretion and interactions with furosemide. J. Pharmacol. Exp. Ther. 222: 237–240

Pan, B. F., Dutt, A., Nelson, A. (1994) Enhanced transepithelial flux of cimetidine by Madin-Darby canine kidney cells overexpressing human P-glycoprotein. J. Pharmacol. Exp. Ther. 270: 1–7

Pritchard, J. B., Miller, D. S. (1993) Mechanisms mediating renal secretion of organic anions and cations. Physiol. Rev. 73: 765–796

Rodvold, K. A., Blum, R. A., Fischer, J. H., Zokufa, H. Z., Rotschafer, J. C., Crossley, K. B., Riff, L. J. (1988) Vancomycin pharmacokinetics in patients with various degrees of renal function. Antimicrob. Agents Chemother. 32: 848–852

Rybak, M. J., Albrecht, L. M., Berman, J. R., Warbasse, L. H., Svensson, C. K. (1990) Vancomycin pharmacokinetics in burn patients and intravenous drug abusers. Antimicrob. Agents Chemother. 34: 792–795

Sokol, P. P. (1991) Mechanism of vancomycin transport in the kidney: studies in rabbit renal brush border and basolateral membrane vesicles. J. Pharmacol. Exp. Ther. 259: 1283–1287

Tsuruo, T., Iida, H., Kitatani, Y., Yokota, K., Tsukagoshi, S., Sakurai, Y. (1984) Effects of quinidine and related compounds on cytotoxicity and cellular accumulation of vincristine and adriamycin in drug-resistant tumor cells. Cancer Res. 44: 4303–4307