Nephrotoxicity of Vancomycin and Drug Interaction Study with Cilastatin in Rabbits

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The nephrotoxic effects of vancomycin hydrochloride (VCM) and the potential drug-drug interaction with cilastatin sodium (CS) were examined in rabbits. The aim of the study was to measure the possible dose-related suppressive effects or elimination by cilastatin of the adverse reactions generated by vancomycin in the kidneys of rabbits. To clarify the interactions of these two drugs, we examined the nephrotoxicity and pharmacokinetics of VCM in the rabbit when administered alone and when coadministered with CS. VCM administered alone (300 mg/kg of body weight as an intravenous bolus; n = 5) caused typical symptoms of nephrotoxicity, such as increases in serum creatinine and blood urea nitrogen (BUN) levels, as well as morphological changes in the kidneys. A lack of such signs of nephrotoxicity was observed in the groups administered VCM plus CS (i.e., CS at 150 mg/kg plus VCM at 300 mg/kg or CS at 300 mg/kg plus VCM at 300 mg/kg, intravenous bolus; n = 5/group). At a reduced combination ratio of VCM plus CS (4:1 ratio, VCM at 300 mg/kg plus CS at 75 mg/kg, intravenous bolus; n = 5) some symptoms of nephrotoxicity induced by VCM were present, but the degree of this effect was much reduced and was significantly different from predose administration values by only modest increases of the BUN and N-acetyl-β-D-glucosaminidase levels (P < 0.05). Overall clearance of VCM was accelerated by coadministration of CS and was found to be dose dependent upon CS. No changes in renal function values from the predose administration values were observed for animals receiving CS alone (300 mg/kg, intravenous bolus; n = 3). These results suggest that CS has the ability to reduce or eliminate in a dose-dependent manner the nephrotoxic effects caused by VCM administration in rabbits.

MATERIALS AND METHODS

This study was approved by the Animal Research Committee of Yamagata University School of Medicine.

Experimental animals. Male Japanese white rabbits (body weight range, 2 to 3 kg) were employed for these studies. Throughout the studies, the animals were housed in an animal room maintained at a temperature of 21 ± 3°C, with lights on for a period of 12 h (6:00 a.m. to 6:00 p.m.). The animals were allowed access to tap water and pelleted feed ad libitum (GC-4; Oriental Yeast Co., Ltd., Tokyo, Japan).

Study drugs. VCM was purchased from Shionogi & Co., Ltd. (Osaka, Japan), and CS was obtained from Banyu Pharmaceutical Co., Ltd. (Tokyo, Japan).

Study protocol. VCM was dissolved in distilled water and administered to the rabbits by injection into an ear vein in a volume of 5 ml/kg of body weight at dosages of 50, 100, 200, and 300 mg/kg. The minimal dose found to clearly cause renal disorder was 300 mg/kg. In addition, since antimicrobial agents are generally prescribed to patients for a 4- to 5-day period, we tried to simulate similar dosing conditions in a preliminary study in which VCM was administered as an intravenous bolus to rabbits in dosages of 50 and 100 mg/kg once per day for 4 consecutive days or in a dosage of 50 mg/kg twice per day for 4 consecutive days. This protocol, however, did not result in any noticeable signs of renal dysfunction. Therefore, in the present study VCM was administered to the rabbits as a single intravenous bolus injection at a dose of 300 mg/kg. The animals were allocated to five groups based upon the following treatment regimens: CS at 75 mg/kg plus VCM, CS at 150 mg/kg plus VCM, CS at 300 mg/kg plus VCM, NaCl at 46 mg/kg (equivalent to the amount of sodium contained in the 300 mg of CS/kg dose) plus VCM, and CS at 300 mg/kg. Each group had five animals except the CS at 300 mg/kg group (three animals). CS and NaCl were dissolved in physiological saline and intravenously administered in a volume of 5 ml/kg at a rate of 2.5 ml/min by using a syringe pump. Immediately thereafter, VCM...
dissolved in distilled water for injection was intravenously administered to the animals in a dosage of 300 mg/5 ml/kg at a rate of 2.5 ml/min in each group except for the CS at 300 mg/kg group. That group received distilled water administered intravenously in a volume of 5 ml/kg following administration of CS at 300 mg/kg. All drug solutions were filter sterilized prior to use by passage through a membrane filter (pore size, 0.22 μm) (Myllex GV; Millipore Products Division, Bedford, Mass.) and then injected into an ear vein.

Urinalysis. Animals were placed in metabolic cages beginning 24 h prior to drug administration, and the exercised urine was collected for the 24-h period prior to administration and then at the 24-h time point after drug administration. The volume of each 24-h pooled urine specimen was measured, and the urinary excretion rate was significantly higher in the CS cotreatment groups than in the NaCl plus VCM group. Postadministration the urinary excretion rate was significantly higher in the NaCl plus VCM group.

Blood and urine biochemical analyses. As shown in Fig. 3, serum creatinine at 24 h after administration was significantly lower in the CS at 150 mg/kg plus VCM group and the CS at 300 mg/kg plus VCM group than in the NaCl plus VCM group. BUN levels at 24 h after drug administration were also significantly lower in the CS cotreatment groups than in the NaCl plus VCM group. Furthermore, the postadministration values for creatinine clearance (CLcr) were significantly higher in the CS at 150 mg/kg plus VCM group, the CS at 300 mg/kg plus VCM group, and the CS at 300 mg/kg plus VCM group than in the NaCl plus VCM group. Postadministration values for urine osmotic pressure and urine potassium concentration were also significantly higher in the CS at 300 mg/kg plus VCM and the CS at 300 mg/kg plus VCM group than in the NaCl plus VCM group. Though no statistically significant differences were found among the five treatment groups with regard to urinary excretions of NAG, significant differences were found between the pre- and postadministration values in the NaCl plus VCM and the CS at 75 mg/kg plus VCM groups.

Drug concentration analyses. The fluorescence polarization immunooassay method (TDx; Dainabot Co., Tokyo, Japan) was used to analyze VCM concentrations. The intraday and interday coefficients of variation for the serum and the urine were each less than 3%, and those for the kidney homogenate were less than 10%. The correlation coefficient of the calibration line for the urine was 0.998 and that for the kidney homogenate was 0.997.

Blood and urine biochemical analyses were conducted using the Astra-8 program (Beckmann K.K., Tokyo, Japan). Statistical analyses. Differences between groups were tested for statistical significance by the multiple comparison test (the Dunnett’s test). Differences between pre- and postadministration values were analyzed using the Student t test. A P value of <0.05 was defined as indicating a significant difference. The pharmacokinetic parameters were calculated with a program for fully automatic analysis of serum concentration data by using the least-squares program MULTI (20). Selection of the optimum model was based on the minimum AIC estimation.

RESULTS

Serum and urine VCM concentrations. Serum VCM concentrations were lower in the CS cotreatment (75 to 300 mg/kg) groups than in the NaCl plus VCM group (Fig. 1). In addition, the summarized data of the pharmacokinetic parameters of VCM (Table 1) indicate that the elimination rate constants (kdt) in the CS cotreatment groups showed statistically significant differences from that of the NaCl plus VCM group. Similarly the areas under the concentration-time curves (AUC) and total clearances (CLs) in the CS at 150 mg/kg plus VCM group and the CS at 300 mg/kg plus VCM group showed statistically significant differences compared with those of the NaCl plus VCM control group. Moreover, as shown in Fig. 2, the urinary excretion rate was significantly higher in the CS cotreatment groups than in the NaCl plus VCM group.

Hematological studies. Blood specimens were collected from an ear vein of the animals at the following time points: prior to each drug administration and at 0.5, 1, 2, 4, 6, 8, and 24 h after completion of the intravenous infusion. Each blood specimen was centrifuged (at 2,000 × g for 10 min), and the concentration of VCM in the supernatant was determined, and the total bilirubin, and total protein levels were determined: the VCM concentration in the urine, urine biochemistry (creatinine, blood urea nitrogen [BUN], potassium, chloride, total bilirubin, and total protein levels) were performed on the serum specimens obtained prior to each drug administration and at 24 h after completion of the administration.

Histological studies. At 24 h after drug administration was complete, the animals were terminated by the administration of thiopental sodium. The kidneys were removed and weighed. One gram of the renal cortex and 1 g of the renal medulla were removed from one kidney, placed in separate 4-ml aliquots of physiological saline, and homogenized by using a Polytron homogenizer (Kinematica AG Littau, Lucerne, Switzerland). Each homogenate was centrifuged (2,000 × g for 10 min), and the concentration of VCM in the supernatant was determined. The pellet was resuspended in 4 ml of physiological saline by stirring and was then centrifuged; this procedure was repeated two more times. The concentration of VCM in each of the supernatants was determined, and the total recovery (86.2 ± 8.8%) was calculated. The contralateral kidney was subjected to light microscopic studies, and a portion was examined by electron microscopy (EM). For the light microscopic studies, tissue specimens were fixed in 10% formaldehyde, dehydrated by the usual method, embedded in paraffin, stained with hematoxylin-eosin, and inspected under a light microscope. For the EM studies, the renal tissue specimen was double fixed with glutaraldehyde and osmium tetroxide and then embedded in epoxy resin. Ultrathin slices were prepared, double stained with uranyl acetate-lead nitrate, and inspected in an electron microscope.

![Graph](http://aac.asm.org/Downloaded_from_page_19.png)

**FIG. 1.** Time course of serum VCM concentration in rabbits. Each value represents the mean for five rabbits (error bars indicate standard deviation). (A) NaCl plus VCM at 300 mg/kg group; (B) NaCl plus VCM at 75 mg/kg plus VCM at 300 mg/kg group; (C) CS at 150 mg/kg plus VCM at 300 mg/kg group; (D) CS at 300 mg/kg plus VCM at 300 mg/kg group. Significant differences from the NaCl plus VCM at 300 mg/kg group are indicated as follows: *P < 0.05; **P < 0.01.

**TABLE 1.** Pharmacokinetic parameters of VCM administered intravenously with NaCl or CS

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>kdt (h⁻¹)</th>
<th>AUC (μg · h/ml)</th>
<th>CL (ml/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl plus VCM</td>
<td>0.165 ± 0.094</td>
<td>6.462 ± 2.725</td>
<td>1.343 ± 0.601</td>
</tr>
<tr>
<td>CS at 75 mg/kg plus VCM</td>
<td>0.326 ± 0.100</td>
<td>2.721 ± 0.558</td>
<td>1.904 ± 0.410</td>
</tr>
<tr>
<td>CS at 150 mg/kg plus VCM</td>
<td>0.536 ± 0.047</td>
<td>1.950 ± 0.284</td>
<td>2.066 ± 0.367</td>
</tr>
<tr>
<td>CS at 300 mg/kg plus VCM</td>
<td>0.611 ± 0.053</td>
<td>3.139 ± 0.282</td>
<td>4.112 ± 0.681</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviation for five rabbits.

* Significantly different from NaCl plus VCM group (P < 0.05).

* Significantly different from NaCl plus VCM group (P < 0.01).
regard to serum creatinine, BUN, CL_{CR}, and urine osmotic pressure, significant differences between pre- and postadministration values were found in the NaCl plus VCM group and, for BUN levels only, the CS at 75 mg/kg plus VCM group. Finally, no significant differences were observed between the CS at 300 mg/kg plus VCM and the CS at 300 mg/kg groups for any of the biochemical parameters examined.

Kidney results (weights, VCM concentrations, and histological findings). At 24 h after administration of VCM, the ratio of the weight of a unilateral kidney to body weight in the NaCl plus VCM group was significantly increased compared with this ratio for the CS cotreatment groups (Table 2). The concentration of VCM in the renal cortex was found to be statistically lower in the CS cotreatment group than in the NaCl plus VCM group, as was the concentration of VCM in the renal medulla. Statistically significant lower levels of VCM were found in the CS at 150 mg/kg plus VCM and the CS at 300 mg/kg plus VCM groups than in the NaCl plus VCM group (Fig. 4). Macroscopic observation of the kidney revealed the renal cortex to have a faded color in the NaCl plus VCM group. Light micrographs taken of the kidney at 24 h after drug administration revealed the presence of dilatation of the lumina of the renal tubules in more than half of the specimens from the NaCl plus VCM group. Kidney specimens of the same group also revealed dilatation of the Bowman’s capsule and images of destruction of renal tubule epithelial cells, as well as the formation of hyaline casts and the presence of degenerated cell fragments in the lumina of the renal tubules. By comparison only very slight formation of hyaline casts in the lumina of the renal tubules was visible in the CS at 150 mg/kg plus VCM, the CS at 300 mg/kg plus VCM, and the CS at 300 mg/kg groups, and no other abnormalities were observed in these groups. Furthermore, examination of kidney specimens by EM revealed the following changes in the NaCl plus VCM group: increased numbers of vacuoles and lysosomes, swollen mitochondria and deformed nuclei in the renal tubule epithelial cells, and destruction of epithelial cells and sloughing off of these cells into the renal tubule lumina (Fig. 5). A high incidence of cellular organelles and hyaline casts in the renal tubule lumina was observed. Again by comparison, although an increased number of lysosomes and cell damage, etc., were also observed in the CS at 300 mg/kg plus VCM group, their incidence and degree were distinctly milder than those found in the NaCl plus VCM group.

DISCUSSION

The clinical importance of vancomycin has been clearly demonstrated. Unfortunately it is also known that vancomycin has the potential to cause nephrotoxicity in the severely ill and in elderly patients; the reported incidence of such adverse reaction is 5 to 10% (1, 2, 9, 12). In the present experimental animal study, we aimed to confirm our preliminary findings that cilastatin has the ability to reduce or inhibit the nephrotoxicity induced by vancomycin in the rabbit (16, 17). Our goal was to investigate the protective effect of cilastatin on vancomycin-induced nephrotoxicity in rabbits.
TABLE 2. Blood and urine biochemical parameters in rabbits

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>K concn</th>
<th>CLCR (ml/min)</th>
<th>NAG (U/g zcr)</th>
<th>Urine osmotic pressure</th>
<th>Kidney wt/body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>0h</td>
<td>2h</td>
<td>4h</td>
<td>0h</td>
<td>2h</td>
<td>4h</td>
</tr>
<tr>
<td>NaCl plus VCM</td>
<td>4.6 ± 0.5</td>
<td>4.2 ± 0.2</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.5</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>d</td>
<td>3.6 ± 0.7</td>
<td>3.0 ± 0.3</td>
<td>3.1 ± 0.3</td>
<td>3.1 ± 0.3</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>CS at 150 mg/kg</td>
<td>3.3 ± 0.5</td>
<td>3.0 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>CS at 300 mg/kg</td>
<td>3.6 ± 0.7</td>
<td>3.0 ± 0.3</td>
<td>2.1 ± 0.3</td>
<td>2.1 ± 0.3</td>
<td>1.7 ± 0.2</td>
</tr>
</tbody>
</table>

Rabbits received VCM and either CS or NaCl as described in the text. Values are means ± standard deviations for five rabbits per treatment group (except for CS at 300 mg/kg (three animals)). Data were recorded before (0 h) and after (24 h) drug administration.

FIG. 4. Concentrations of VCM in the rabbit renal cortex and medulla 24 h after the administration of VCM (300 mg/kg) to rabbits. Each value represents the mean for five rabbits (error bars indicate standard deviation). Significant differences from the NaCl plus VCM at 300 mg/kg group are indicated as follows: *, P < 0.05; **, P < 0.01.

was to determine whether a dose-dependent reduction or elimination of the VCM-induced nephrotoxicity in rabbits could be achieved with the coadministration of cilastatin.

Clear evidence indicative of renal impairment was quickly established in this model at 24 h after administration of VCM in saline (NaCl at 46 mg/kg plus VCM at 300 mg/kg). Evidence of a dose-related prevention by cilastatin of this VCM-induced renal impairment was demonstrated with the coadministration of CS at 150 or 300 mg/kg. Also, the CS at 300 mg/kg (plus distilled water) control group showed no abnormalities in the clinical laboratory tests. Histological changes in the kidneys of animals in these latter groups were very slight. Whereas animals in the CS at 75 mg/kg plus VCM at 300 mg/kg group (4:1 ratio) showed some symptoms of nephrotoxicity induced by VCM, the degree of the nephrotoxic symptoms was distinctly reduced in comparison with that of the NaCl plus VCM at 300 mg/kg control group. Previous dose-ranging studies established that CS at 30 mg/kg plus VCM at 300 mg/kg (10:1 ratio) resulted in a degree of nephrotoxic symptoms equivalent to that produced by CS at 75 mg/kg plus VCM at 300 mg/kg.

The mechanism of VCM-induced nephrotoxicity remains unclear. In our attempts to explain the mechanism of the effect of CS in lessening and/or eliminating the VCM-induced nephrotoxicity in this experimental animal model, we assumed as one possible pathway the inhibition of VCM uptake by renal cortex cells. CS is known to eliminate the nephrotoxic potential of IPM, which capacity led to the development of the fixed combination antibiotic IPM-CS used in clinical practice. It has been postulated that this nephrotoxicity-alleviating activity of CS is not solely due to its inhibitory action on the renal dipeptidase, dehydropeptidase-I (DHP-I), residing in the brush border membrane of the proximal tubule, but also has an (dual) inhibitory effect on the uptake of IPM by the epithelial cells of the proximal renal tubules (4).

It has also been reported that CS will reduce the concentration of cephaloridine, which is not metabolized by DHP-I in the renal cortex, to 1/6 of the (normal) level observed when CS is not coadministered. This reduction was sufficient to suppress the manifestation of nephrotoxicity in response to cephaloridine administration (6). Results of our present experiment...
show the concentration of VCM in the renal tissues of the NaCl plus VCM group animals to be markedly higher than the VCM concentration in the CS concomitant treatment groups. The renal clearance of VCM was correlated with the total clearance of VCM (correlation coefficient, 0.979). As previously reported, VCM undergoes renal tubular secretion in the rat (10). Earlier reports from this laboratory demonstrated a reduction of VCM nephrotoxicity in rabbits by IPM-CS, FMOX, and fosfomycin sodium, but no lessening effects were observed by coadministration of CAZ or CPIZ. It has been reported that CS (14) and FMOX (7) are excreted by renal tubular secretion, whereas CAZ (13) and CPIZ (15) are minimally excreted by this route. The potential reduction of the renal concentration of VCM by CS may be due to the interaction of CS with renal tubular secretion or reabsorption of VCM.

Other possible mechanisms involved in this protective phenomenon are such events as glomerular impairment, interstitial nephritis due to a hypersensitivity reaction, or obstructive renal impairment. There is also the possibility of prerenal renal failure due to a decrease in the renal blood flow. Wold and Turnipseed (18) reported that during administration of a 5% VCM solution at a dosage of 20 mg/kg to dogs by intravenous infusion at a rapid rate of 15 mg/min, the mean arterial blood pressure decreased by 40%. This suggests the possibility that renal impairment may be caused by a decrease in blood pressure. In our present study, however, concomitant administration of CS with VCM suppressed the manifestation of renal
impairment in the rabbits. Earlier reports show that the mean arterial blood pressure decreased slightly when dogs were placed under anesthesia with sodium pentobarbital and CS, at a concentration of 100 mg/kg, was administered (8). In light of this finding, it seems unlikely that CS prevents a blood pressure decrease triggered by VCM and thereby would prevent the manifestation of renal impairment caused by VCM in this rabbit model.

In conclusion, based on the observations of this study, it was confirmed that CS at dose-dependent levels has the ability to reduce and eliminate development of VCM-induced nephrotoxicity in rabbits. Documentation of a similar beneficial response in human patients remains a worthwhile goal.

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REFERENCES