
Regular Article

Decreased Renal Accumulation and Toxicity of a New VCM Formulation in Rats with Chronic Renal Failure

Naoko HODOSHIMA1,2, Satoshi MASUDA1 and Ken-ichi INUI1,*

1Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto, Japan
2MEIJI SEIKA KAISHA, LTD., Product Management & Promotion Dept., Tokyo, Japan

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: We previously reported that MEEK, a generic product of vancomycin hydrochloride (VCM), was less nephrotoxic than a conventional preparation (S-VCM) in normal rats at a nephrotoxic dose (400 mg/kg) of VCM.1) To infer the clinical significance of this finding, we compared the risk of nephrotoxicity of these two formulations in rats with chronic renal failure in this study.

MEEK or S-VCM was given intravenously to two weeks post-5/6 nephrectomy rats, and the pharmacokinetic profile of VCM and pathological evaluation were compared. There were no differences at the daily clinical dose (40 mg/kg), but at the twice the daily clinical dose (80 mg/kg), the mean plasma concentration of VCM was higher after S-VCM administration than after MEEK and the CLtot and CLr decreased to approximately 60% of those after MEEK. The renal tissue concentration of VCM was 1.5-fold higher at 24hr after S-VCM administration than after MEEK. Pathologically, no marked differences between the findings were observed at 24hr after administration of each formulation. These findings suggest that MEEK reduces renal damage caused by VCM and prevents the iatrogenic aggravation of nephrotoxicity. These results hold out hope that MEEK will permit high-dose administration of VCM, while revealing clinical significance of the nephrotoxicity-reduction by MEEK.

Key words: vancomycin; nephrotoxicity; drug-induced nephropathy; D-mannitol; Macrogol400; 5/6 nephrectomy

Introduction

Vancomycin hydrochloride (VCM) is a glycopeptide antibiotic that was isolated from Amycolatopsis orientalis by Eli Lilly & Co. and is especially well regarded for its excellent antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA).2) While VCM has outstanding therapeutic efficacy, it also causes side effects such as nephropathy and red neck syndrome. Therefore, therapeutic drug monitoring is required.3–5)

In Japan, an oral preparation of VCM was approved in 1981, after which a parenteral formulation of VCM was approved in 1991 for MRSA infections. The procedure for reconstituting the parenteral formulation of VCM is complicated by the physicochemical characteristics of the drug that readily give rise to the formation of a gel during reconstitution.6,7) In response to the clinical need, formulation studies were undertaken and led to the development of VCM for intravenous infusion (MEEK), a lyophilized preparation containing 100 mg each of D-mannitol and Macrogol400 per 500 mg of VCM, which was introduced in 2002.8,9)

During the development of MEEK, we performed safety studies of this drug in rats and discovered that these additives reduced the nephrotoxicity of high-dose VCM (400 mg/kg) in normal rats.1) Because VCM-induced nephrotoxicity is associated with the extent of exposure, VCM therapy has a relatively high risk of causing progressive renal failure in patients with impaired renal function. If much of the effect of the additives on the nephrotoxicity of VCM was due to inhibition of drug accumulation in the kidneys, their effect ought to be more conspicuous in the presence of decreased renal function. If the renal accumulation of VCM can be reduced by modifying the formulation, administration of the drug at higher doses could facilitate the elimination of infections with greater safety. Based on this background, we hypothesized that VCM-induced progressive renal dysfunction could be avoided in chronic renal failure by administering MEEK rather than the
standard formulation (S-VCM) due to less renal accumulation of the drug. The present study was undertaken to verify this finding and to reason its clinical significance by conducting pharmacokinetic and pathophysiological analyses in 5/6 nephrectomized rats, an animal model of chronic renal failure.10–12)

Materials and Methods

The test substances: Commercially available VCM for IV infusion 0.5 MEEK (Kobayashi Kako Co., Ltd. and Meiji Seika Kaisha, Ltd., Fukui and Tokyo; MEEK) and VCM hydrochloride for IV infusion 0.5 g (Shionogi & Co., Ltd., Osaka; S-VCM) were used in this study. For each 500 mg of VCM, MEEK contains 100 mg each of D-mannitol and Macrogol400.

Animals and treatment: Six-week-old male SD rats (weighing approximately 200 g, Japan SLC. Inc.) were used to produce 5/6 nephrectomized (5/6Nx) rats according to the usual method.10–12) For two weeks after surgery, the animals were offered food and water ad libitum and then were used in the pharmacokinetic study. During the experimental period, the animals were provided with pellets for laboratory animals (Oriental Yeast Co., Ltd.). The animal experiments were performed in accordance with the Guidelines for Animal Experiments of Kyoto University.

The 5/6Nx rats were produced as follows: After being fasted for about 16 hours, each rat was anesthetized with Nembutal (weighing approximately 200 g, Japan SLC. Inc.) to prepare dosing solutions containing VCM at a concentration of 4 or 8 mg/mL. The test substances were administered after the following procedure on the animals: Each animal was weighed and anesthetized with ether. Then a polyethylene tube (Becton Dickinson Co., Ltd.; PE50) was inserted into the femoral artery, a syringe was sutured to the external urethral meatus, and the animal was immobilized in a Bellman cage. After the animals had regained consciousness, the test substances were administered. The dosing volumes were calculated on the basis of the body weight measured immediately before dosing, and the test substance was administered intravenously in a volume of 10 mL/kg via the tail vein at a rate of 2 mL/min.

Blood was collected into heparinized sample tubes from the polyethylene tube inserted into the femoral artery immediately before the intravenous administration, and at 5, 15, and 30 minutes, and 1, 3, and 6 hours post-administration. Blood samples were promptly centrifuged for 10 minutes at 4°C and 5,000 r.p.m. (1,700 × g) to separate plasma. Spontaneously voided urine was collected on the day before administration, as well as for 0–6 hours and 6–24 hours after administration. The rats in the 80 mg/kg group in the second part of the study were killed by exsanguination by cutting the axillary arteries immediately after blood was collected at 24 hours post-administration, and their kidneys were removed. The kidneys intended for pathological examination were weighed and analyzed with a fluorochrome polarization immunoassay (FPIA) with a TDX analyzer*, using the TDX™ [VCM Abbott Kit (Abbott Japan Co., Ltd.)]. The concentration range of the assay was 2–100 μg/mL, and the samples were diluted using purified water. Furthermore the renal concentration of VCM at 24 hours after administration of each

Preparation of dosing solutions: The test substances were dissolved by adding physiological saline JP (saline) to prepare dosing solutions containing VCM at a concentration of 4 or 8 mg/mL.

Pharmacokinetic study: We previously revealed that no significant differences were observed between MEEK and S-VCM in the pharmacokinetic data after these two formulations were given to normal rats at the daily clinical dose (40 mg/kg as VCM).13) Therefore, in the present study, MEEK was given to Sham and 5/6Nx rats at a dose of 40 mg/kg to compare the pharmacokinetic profiles of VCM between these animals. Subsequently, MEEK or S-VCM was given to 5/6Nx rats as a single dose of 40 or 80 mg/kg, and the pharmacokinetic profiles of the two formulations were compared for each dose. Since differences were noted in the blood concentration of VCM between the two formulations at a dose of 80 mg/kg, the kidneys of the animals were removed at 24 hours after administration of each test substance at this dose, and the renal tissue concentrations of VCM were compared.

The test substances were administered after the following procedure on the animals: Each animal was weighed and anesthetized with ether. Then a polyethylene tube (Becton Dickinson Co., Ltd.; PE50) was inserted into the femoral artery, a syringe was sutured to the external urethral meatus, and the animal was immobilized in a Bellman cage. After the animals had regained consciousness, the test substances were administered. The dosing volumes were calculated on the basis of the body weight measured immediately before dosing, and the test substance was administered intravenously in a volume of 10 mL/kg via the tail vein at a rate of 2 mL/min.

Blood was collected into heparinized sample tubes from the polyethylene tube inserted into the femoral artery immediately before the intravenous administration, and at 5, 15, and 30 minutes, and 1, 3, and 6 hours post-administration. Blood samples were promptly centrifuged for 10 minutes at 4°C and 5,000 r.p.m. (1,700 × g) to separate plasma. Spontaneously voided urine was collected on the day before administration, as well as for 0–6 hours and 6–24 hours after administration. The rats in the 80 mg/kg group in the second part of the study were killed by exsanguination by cutting the axillary arteries immediately after blood was collected at 24 hours post-administration, and their kidneys were removed. The kidneys intended for pathological examination were weighed and analyzed in 10% neutral-buffered formalin. The plasma, urine, and kidney samples for analysis of drug concentrations were stored frozen at −20°C or below.

Analysis of drug concentrations: The VCM concentration in the plasma and urine was analyzed by a fluorescence polarization immunoassay (FPIA) with a TDX analyzer*, using the TDX™ [VCM Abbott Kit (Abbott Japan Co., Ltd.)]. The concentration range of the assay was 2–100 μg/mL, and the samples were diluted using purified water. Furthermore the renal concentration of VCM at 24 hours after administration of each
test substance at 80 mg/kg dose was determined. The kidneys were weighed, 9 times as much saline as the kidney weight was added, and a 10% homogenate was prepared using a Polytron-Aggregate PCU/11 homogenizer (KINEMATICA AG, Littan-Lucerne, Switzerland). The sample was further diluted with saline, and then analyzed with the same FPIA used for plasma and urine. The concentration range for the assay was 5–50 μg/mL.

**Pharmacokinetic analysis:** The time course of the plasma VCM concentration was analyzed by a model-independent method using WinNonlin (Scientific Consulting, Inc., Ver.3.3). The pharmacokinetic parameters determined were the area under the plasma concentration-time curve (AUC_{0–obs} and AUC_{0–∞}), terminal half-life (T_{1/2}), total body clearance (CL_{tot}), and the volume of distribution at steady state (V_{dss}). The renal clearance (CL_{r}) and non-renal clearance (CL_{nr}) were calculated using the following equations.

\[
\begin{align*}
CL_{tot} &= \frac{\text{Dose}}{\text{AUC}_{0–∞}} \\
CL_{r} &= \frac{X_{0–∞}}{\text{AUC}_{0–obs}} (X: \text{total urinary excretion}) \\
CL_{nr} &= CL_{tot} – CL_{r} 
\end{align*}
\]

**Renal function tests and pathological examination:** Blood urea nitrogen (BUN) concentration and creatinine clearance (C_{cr}) were used as the markers of renal function. Briefly, BUN and plasma creatinine concentrations (P-C_{re}) were measured in the Sham and 5/6Nx rats before administration, and the concentration of creatinine in the pooled urine (U-C_{re}) obtained from the rats over 24 hours before administration was measured. In the 5/6Nx rats receiving each test substance at a dose of 40 or 80 mg/kg, the BUN, P-C_{re}, and U-C_{re} were also measured after administration. BUN was measured using a Urea Nitrogen B Test Wako (Wako Pure Chemical Industries, Ltd., Osaka), while P-C_{re} and U-C_{re} were measured using a Creatinine Test Wako (Wako Pure Chemical Industries). C_{cr} was calculated from the P-C_{re}, U-C_{re}, and urine volume (urine volume was determined with the specific gravity being defined as 1) by the following equation.

\[
C_{cr} = \frac{Y_{0–24}}{(P-C_{re} \times 24) / 60} (\text{mL/min})
\]

(Y_{0–24}: \text{total U-C}_{re})

The kidneys of the Sham and 5/6Nx rats were examined pathologically before administration, while the kidneys of the 5/6Nx rats were examined at 24 hours after receiving each test substance at a dose of 80 mg/kg. Light microscopy was done on samples fixed in 10% neutral-buffered formalin and stained with hematoxylin and eosin (H-E) according to the routine method.

**Statistical analysis:** The renal function data (BUN and C_{cr}) obtained from the Sham and 5/6Nx rats before drug administration and the pharmacokinetic parameters determined after the administration of MEEK at 40 mg/kg to Sham and 5/6Nx rats were statistically analyzed for differences using Student’s t-test (the level of significance was set at p<0.05). Two-way analysis of variance (ANOVA), with the test substance and dose selected as factors, was also performed for the CL_{tot}, CL_{r}, CL_{nr}, V_{dss}, and urinary excretion rate obtained after administration of MEEK or S-VCM at 40 or 80 mg/kg to 5/6Nx rats. All statistical analyses were done using SAS software (SAS Institute Japan, Ver. 8).

In the present experiment, there were 6 or less animals per group, because 2 groups of the same batch were compared at the same time, and thus it was technically difficult to handle many animals.

**Results**

**Renal function and pathological findings in the 5/6Nx rats:** The renal function data obtained from the Sham and 5/6Nx rats are shown in Table 1. In the 5/6Nx rats, C_{cr} decreased to about 22% of the value in the Sham rats and BUN increased to about 3-fold the level in the Sham rats, and significant differences were noted. No significant difference was found in the urine volume, but the mean value was about 2-fold the level in the Sham rats. Light microscopic examination of the hematoxylin-and-eosin-stained kidney samples revealed histopathological changes caused by an increased interventional load and exhaustion in those of the 5/6Nx rats compared with those of the Sham rats. The changes included hypertrophy of the glomeruli, enlargement of the mesangial region, dilatation of the renal tubules, and inflammatory cell infiltration into the interstitium (Fig. 1).

**Pharmacokinetics:** Figures 2 and 3 show the profiles of the plasma concentration of VCM after the administration of MEEK or S-VCM to the 5/6Nx rats at a dose of 40 or 80 mg/kg. Figure 2 also shows the profile of the mean plasma concentration in the Sham rats. The pharmacokinetic parameters obtained from these data on the plasma concentration and cumulative urinary excretion

**Table 1. Renal function data in rats**

<table>
<thead>
<tr>
<th>Renal function</th>
<th>Sham</th>
<th>5/6Nx</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Body weight</td>
<td>299.3±20.2</td>
<td>258.8±26.2</td>
</tr>
<tr>
<td>Urine volume</td>
<td>16.4±3.5</td>
<td>30.8±12.5</td>
</tr>
<tr>
<td>P-C_{re}</td>
<td>0.31±0.04</td>
<td>0.96±0.09**</td>
</tr>
<tr>
<td>U-C_{re}</td>
<td>88.7±21.1</td>
<td>37.1±18.8*</td>
</tr>
<tr>
<td>C_{cr}</td>
<td>3.87±0.98</td>
<td>0.85±0.11**</td>
</tr>
<tr>
<td>BUN</td>
<td>15.6±2.7</td>
<td>46.2±3.9**</td>
</tr>
</tbody>
</table>

*(mean ± S.D.)*

*: Significant difference from the Sham-operated group (p<0.05)

**: Significant difference from the Sham-operated group (p<0.01)
Fig. 1. Hematoxylin and eosin staining of renal cortex obtained from sham-operated (a) and 5/6Nx rats (b). The magnification for both pathograph is x50.

Fig. 2. Plasma concentration of VCM (40 mg/kg) after intravenous administration of MEEK to sham-operated rats (Sham) or 5/6 nephrectomized rats (5/6Nx), and S-VCM to 5/6Nx rats. MEEK (open circle, closed triangle) or S-VCM (closed circle) was administered to sham-operated (closed triangle) or 5/6Nx (open circle, closed circle) rats as a dose of 40 mg/kg of VCM (10 mL/kg) at a rate of 2 mL/min via tail vein. Blood samples were collected from the left femoral artery at 0.083, 0.25, 0.5, 1, 3 and 6 hour after the injection. Each point represents the mean ± S.D. of four rats. For reference, the profiles (open square, closed square) of the plasma concentrations (40 mg/kg) in normal rats.1)

Fig. 3. Plasma concentration of VCM (80 mg/kg) after intravenous administration of MEEK or S-VCM to 5/6Nx rats. MEEK (open circle) or S-VCM (closed circle) was administered to 5/6Nx rats as a dose of 80 mg/kg of VCM (10 mL/kg) at a rate of 2 mL/min via tail vein. Blood samples were collected from the left femoral artery at 0.083, 0.25, 0.5, 1, 3 and 6 hour after the injection. Each point represents the mean ± S.D. of six rats for MEEK treated group and five rats for S-VCM treated group, respectively. *p < 0.05; **p < 0.01, significantly different from MEEK treated rats. Sham, sham-operated rats; 5/6 Nx, 5/6 nephrectomized rats.

Pharmacokinetic profiles were compared after MEEK was given to 5/6Nx rats and Sham rats at a dose of 40 mg/kg. It was found that in the 5/6Nx rats the $T_{1/2}$ was about 2.4-fold longer and the AUC$_{0-\infty}$ was about 2.6-fold greater than in the Sham rats, while the CL$_{tot}$ decreased to about 40% of that in the Sham rats. In the 5/6Nx group, the cumulative urinary excretion rate decreased to about 85% and the CL$_e$ decreased to about 30% of the level in the Sham rats. There were significant differences in these parameters, but there were no significant differences in the Vd$_s$ or the CL$_nr$ between the
Table 2. Pharmacokinetic parameters and urinary excretion of VCM after intravenous administration of MEEK or S-VCM to Sham-operated or 5/6Nx rats

<table>
<thead>
<tr>
<th>PK parameters</th>
<th>Sham</th>
<th>5/6Nx MEEK</th>
<th>5/6Nx S-VCM</th>
<th>5/6Nx MEEK</th>
<th>5/6Nx S-VCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>0.68±0.08</td>
<td>1.65±0.34**</td>
<td>1.85±0.39</td>
<td>1.58±0.18</td>
<td>2.76±1.06*</td>
</tr>
<tr>
<td>AUC0—(µg·h/mL)</td>
<td>97±10</td>
<td>255±50**</td>
<td>315±69</td>
<td>485±49</td>
<td>824±255*</td>
</tr>
<tr>
<td>Urinary excretion (% of dose, 0–24 h)</td>
<td>98.0±4.9</td>
<td>83.9±7.6*</td>
<td>87.1±13.6</td>
<td>86.7±5.8</td>
<td>83.7±9.0</td>
</tr>
<tr>
<td>CLtot (mL/min/kg)</td>
<td>6.88±0.70</td>
<td>2.67±0.59**</td>
<td>2.17±0.58</td>
<td>2.75±0.29</td>
<td>1.77±0.56**</td>
</tr>
<tr>
<td>Vdss (mL/kg)</td>
<td>322.5±43.5</td>
<td>327.5±15.0</td>
<td>300.0±8.2*</td>
<td>328.3±26.4</td>
<td>344.0±50.8</td>
</tr>
<tr>
<td>CLr (mL/min/kg)</td>
<td>6.75±0.44</td>
<td>2.25±0.73**</td>
<td>1.88±0.44</td>
<td>2.36±0.33</td>
<td>1.47±0.62*</td>
</tr>
<tr>
<td>CLnr (mL/min/kg)</td>
<td>0.17±0.33</td>
<td>0.42±0.21</td>
<td>0.34±0.30</td>
<td>0.39±0.13</td>
<td>0.30±0.01</td>
</tr>
</tbody>
</table>

*(mean±S.D.)*

*, **: p<0.05 and p<0.01 significant difference from the Sham-operated group (40 mg/kg)
†: p<0.05 significant difference from 5/6Nx after the administration of MEEK (40 mg/kg)
*, **: p<0.05 and p<0.01 significant difference from 5/6Nx after the administration of MEEK (80 mg/kg)

Two groups (Fig. 2, Table 2). Pharmacokinetic profiles were compared after the two formulations were given to 5/6Nx rats. At a dose of 40 mg/kg, the mean plasma concentration of VCM was slightly higher after the administration of S-VCM than after the administration of MEEK, but there were no significant differences in the AUC0—, CLtot, or CLr between these two formulations (Fig. 2, Table 2). In contrast, at a dose of 80 mg/kg, the mean plasma concentration of VCM was significantly higher after the administration of S-VCM than after that of MEEK; after the administration of S-VCM, the T1/2 was about twice as long and the AUC0— was also approximately 2-fold greater than after the administration of MEEK, while the CLtot and CLr decreased to approximately 60% of those after the administration of MEEK (Fig. 3, Table 2). The CLr, Vdss, and urinary excretion rate in the 5/6Nx rats were analyzed by two-way ANOVA with the test substance and dose selected as factors, and no interaction was seen between the two factors for any of the parameters (p>0.15). There were no significant differences in any of the pharmacokinetic parameters between the doses, while there were significant differences with respect to CLtot (p=0.0040) and CLr (p=0.0144) between MEEK and S-VCM at a dose of 80 mg/kg.

The renal drug concentration was measured at 24 hours after the administration of each test substance to the 5/6Nx rats at 80 mg/kg. It was found that the renal tissue concentration of VCM was about 1.5-fold higher after the administration of S-VCM (653.8±186.0 µg/g) than after the administration of MEEK (442.9±120.6 µg/g) (p=0.0490, Fig. 4). At 24 hours after administration, the plasma concentration of VCM was below the lower limit of quantitation (2 µg/mL) after administration of either substance.

Fig. 4. Renal tissue concentration of VCM after intravenous administration of MEEK or S-VCM as a dose of 80 mg/kg of VCM to 5/6Nx rats. Twenty-four hours after the intravenous administration of MEEK or S-VCM as a dose of 80 mg/kg of VCM via tail vein, the kidneys were collected for the measurement of renal tissue concentration of VCM. Dots indicate the concentrations of VCM in the remnant kidneys after the administration of MEEK or S-VCM.*p<0.05, significantly different from MEEK-treated group.

Nephrotoxicity: Pathological examination revealed slight hypertrophy of the glomeruli, slight enlargement of the mesangial region, and slight dilatation of the renal tubules after the administration of S-VCM, but showed no marked differences between the findings after administration of the two formulations (Fig. 5). Changes in the renal function were assessed based on the BUN and Cr levels obtained before and after administration, and no significant differences were noted (Table 3).
Fig. 5. Hematoxylin and eosin staining of renal cortex obtained from 5/6Nx rats at 24 hours after the intravenous administration of MEEK (a) or S-VCM (b) as a dose of 80 mg/kg of VCM. The magnification for both pathograph is x25.

Table 3. Creatinine clearance and BUN before and after intravenous administration of MEEK or S-VCM to 5/6Nx rats

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Product</th>
<th>n</th>
<th>Ccr (mL/min)</th>
<th>BUN (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>before</td>
<td>after</td>
</tr>
<tr>
<td>40</td>
<td>MEEK</td>
<td>4</td>
<td>0.78 ± 0.65</td>
<td>0.97 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>S-VCM</td>
<td>4</td>
<td>0.74 ± 0.33</td>
<td>0.99 ± 0.21</td>
</tr>
<tr>
<td>80</td>
<td>MEEK</td>
<td>6</td>
<td>1.20 ± 0.33</td>
<td>1.20 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>S-VCM</td>
<td>5</td>
<td>0.84 ± 0.37</td>
<td>0.71 ± 0.26</td>
</tr>
</tbody>
</table>

Ccr: creatinine clearance
BUN: blood urea nitrogen

Discussion

Because 5/6Nx rats have pathological characteristics that resemble those of renal failure in humans, this animal model has been widely used for investigating pharmacokinetics in renal impairment. Since the residual glomeruli in 5/6Nx rats are originally normal, this model involves less variation among animals. Thus, 5/6Nx rats, after a long period of housing, have been used as a model of non-inflammatory chronic renal failure.10–12) The 5/6Nx rats model has already been used to assess changes in the expression of drug transporters localized in the kidneys under pathological conditions, and various findings are being accumulated at the molecular or cellular level concerning the mechanisms of pharmacokinetic regulation.13,14) A recent report has shown that at 2 weeks after 5/6Nx, the expression of OCT1, OAT1, OAT3, and PEPT1 in the kidneys are maintained as tubular drug transporters.15,15–17) That 5/6Nx rats have decreased levels of expression of rMATE1 and NHE3.18) Considering that VCM is excreted mainly in urine and that the pattern of urinary excretion of VCM is similar between humans and rats, 5/6Nx rats seems to be a good model for investigating the clinical significance of the antinephrotoxic effect of the additives contained in MEEK in rats.

The 5/6Nx model is characterized by decreased Ccr and increased BUN values, which are observed during renal impairment. These features suggest the occurrence of hypertrophy of the remnant glomeruli and injury in the remnant glomeruli due to loss of nephrons. These findings confirmed the appropriateness of 5/6Nx rats as a model of renal damage (Fig. 1). If the pathological state of 5/6Nx rats is viewed as analogous to that of humans (the Ccr of healthy adults is defined as ≥70 mL/min19)), the renal function of 5/6Nx rats seems to be equivalent to severe renal impairment (Ccr ≤30 mL/min) when calculated from the ratio of the Ccr in the Sham rats to that in the 5/6Nx rats.

We previously revealed that no significant differences were observed between MEEK and S-VCM in the pharmacokinetic data or renal function after these two formulations were administered to normal rats at the daily clinical dose.1) Then, in the present study we compared the pharmacokinetics of VCM using MEEK in 5/6Nx rats with those in Sham rats. In the 5/6Nx rats, the CLr was lower in a Ccr-dependent manner, the plasma concentration was higher, and elimination from the plasma was slower than in the Sham rats. These findings seem to represent the pharmacokinetics of VCM, in which a high correlation was shown between Ccr and the elimination rate constant.20) The cumulative urinary excretion rate was decreased at 24 hours after administration. This finding seems to be due to delayed excretion of VCM associated with decreased CLr. The profiles of the mean blood concentration in the normal rats are shown for reference. Comparison between this profile and that of the Sham rats revealed differences. These differences were probably caused by the influence of the sham operation and that of two weeks of housing under that condition. These differences seem to include those caused by the age or batch of the animals. However, they are
much smaller than those in the mean plasma concentration between the 5/6Nx rats and the Sham rats. In the present experiment, one dose level was used to assess the influence of treatment on the Sham group, because 2 groups of the same batch were compared at the same time, and thus it was technically difficult to handle many animals.

In the 5/6Nx rats, no significant differences were observed in the pharmacokinetic data between MEEK and S-VCM after these two formulations were administered at the daily clinical dose (40 mg/kg), while differences were noted in the pharmacokinetics after the two substances were administered at a dose of 80 mg/kg; the renal tissue concentration of VCM was about 1.5-fold higher after the administration of S-VCM than after the administration of MEEK. At this time, it was difficult to detect the difference in the exacerbation of renal impairment based on the results of pathological examination after administration of the two formulations or on the changes in the renal function before and after their administration (Fig. 5, Table 3). Based on these findings, use of 5/6Nx rats seems to have enabled us to detect pharmacokinetic differences between the two formulations under conditions where there is less histopathological influence of the drugs. Therefore, since the test substances were administered once at the approved clinical dose in the present study, it seems that this dose did not provide sufficient exposure to cause nephrotoxicity of VCM histopathologically and that the additives in MEEK had some influence on the renal distribution and process of excretion of VCM. In order to elicit histopathological differences in this model after administration of the two formulations, several days of multiple-dose administration may be considered, but this would make it more difficult to determine whether the differences are caused by the progression of the clinical condition in this model or by the drugs. Thus, there seems to be limitations in investigation using this model. Assuming that the additives in MEEK reduce the nephrotoxicity of VCM mainly by decreasing renal accumulation of VCM, MEEK will prevent further drug-induced nephrotoxicity and will enable dose-escalation of VCM. This is expected to provide the clinical significance of the nephrotoxicity-reducing effect of MEEK.

The nephrotoxicity of VCM has been reported in toxicological evaluation in cultured epithelial cells (LLC-PK1 cells), in investigation of the mechanisms of renal excretion using ion channel drugs, with respect to the protective effect of fosfomycin and imipenem-cilastatin against the nephrotoxicity of VCM, and with respect to reduction of the uptake of VCM into kidney cells. However, the mechanisms of the nephrotoxicity of VCM still remain unclear, and no membrane transporters, which control the transfer of VCM from the blood to tubular epithelial cells, have been found at present. The mechanism leading to the reduced nephrotoxicity of VCM due to the additives in MEEK is also unknown. Assuming that the additives in MEEK reduce the nephrotoxicity of VCM mainly by decreasing renal accumulation of VCM, the mechanisms may be related to the cytoprotective effect of D-mannitol and to the changes in the renal distribution of VCM due to increases in renal blood flow and the glomerular filtration rate. Further, pharmacokinetic and pathophysiological comparison between MEEK and S-VCM will provide information concerning the involvement of the mechanisms of pharmacokinetic regulation at the molecular or cellular level in the effects of the additives on the pharmacokinetics of VCM.

In recent years, the efficacy, toxicity, and blood levels of VCM have been discussed in relation to the design of a regimen for VCM from the perspective of TDM, and peak and trough values have been discussed as to their appropriateness. To achieve the efficacy of VCM with a high probability, it is necessary to design a regimen for VCM in such a way as to increase its AUC while guarding against adverse reactions. For this purpose, it is necessary to increase the daily dose while paying attention to safety, particularly trough values. It has been suggested that a time-dependent decrease of VCM clearance is one of the factors that interferes with the precision of estimation by the Bayesian method during the treatment period. These findings and the results of the present study suggest that delayed CL of VCM may be caused by accumulation of the drug itself in the kidneys. Therefore, VCM-induced nephrotoxicity may be avoided by the use of other drugs that decrease the renal distribution of VCM, such as fosfomycin and imipenem-cilastatin, or by a new formulation, such as MEEK, which contains additives that will reduce accumulation of the drug in the kidneys.

We used 5/6Nx rats in the present study, which only produced results in a limited experimental system and thus has limitations in inferring various clinical symptoms actually encountered. Therefore, a prospective clinical study should be designed to clarify the clinical significance of MEEK with respect to VCM-induced renal damage. It is hope that pharmacological and pathophysiological research will progress further.

Acknowledgments: This work was supported in part by a grant-in-aid for Research on Advanced Medical Technology from the Ministry of Health, Labor and Welfare of Japan, by the Japan Health Science Foundation "Research on Health Sciences Focusing on Drug Innovation", by a grant-in-aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan, and by the 21st Century COE program "Knowledge Information Infrastructure for Genome
References


7) Package Insert, VANCOMYCIN

8) Package Insert, VANCOMYCIN for I.V. Infusion 0.5 [MEEK]


