Regular Article

Protective Effect of Inactive Ingredients against Nephrotoxicity of Vancomycin Hydrochloride in Rats

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Summary: A generic form of vancomycin for I.V. infusion (MEEK) is more soluble and stable than the brand-name form of vancomycin hydrochloride (VCM) due to the addition of two inactive ingredients: D-mannitol and Macrogol400 (PEG400). The aim of the present study was to compare the nephrotoxicity of MEEK with that of brand-name VCM (S-VCM) and to analyze the pharmacokinetics of these preparations.

Following administration to rats at the clinical dose of 40 mg/kg, there was no difference between MEEK and S-VCM with regard to pharmacokinetics and effects on the kidneys, indicating that MEEK should be as effective as S-VCM. When administered at the nephrotoxic dose of 400 mg/kg, S-VCM caused impairment of renal function and kidney damage, and an increase of the plasma concentration due to decreased renal clearance was observed. In contrast, MEEK had virtually no effect on renal function or the kidneys and did not cause a marked change of renal clearance. These findings suggest that the inactive ingredients in MEEK play a role in reducing the nephrotoxicity of VCM.

Key words: vancomycin; nephrotoxicity; D-mannitol; PEG400; renal clearance

Introduction

Vancomycin hydrochloride (VCM) is a glycopeptide antibiotic that was isolated from Streptomyces orientalis by Eli Lilly & Co. and shows a superior antibacterial effect against multidrug-resistant methicillin-resistant Staphylococcus aureus. Because VCM is mainly excreted by the kidneys and is also nephrotoxic, therapeutic drug monitoring is needed to ensure appropriate clinical use.1-5 Last year, vancomycin for intravenous infusion was released by Meiji Seika Kaisha, Ltd. and Kobayashi Kako Co., Ltd. as a generic drug under the trade name of VANCOMYCIN for I.V. Infusion 0.5 'MEEK' (hereafter referred to as MEEK), whereas the brand-name form of VCM (S-VCM) does not contain any inactive ingredients, MEEK contains 100 mg each of D-mannitol and Macrogol400 (PEG400) combined with VCM (500 mg activity) to improve solubility and stability. The aim of the present study was to compare nephrotoxicity, a significant adverse reaction to VCM, between MEEK and the brand-name form of VCM (S-VCM), and to analyze the pharmacokinetics of both drugs in relation to the pathophysiology after intravenous administration.

Materials and Methods

Test substances: Commercially available VANCOMYCIN for I.V. Infusion 0.5 'MEEK' (Meiji Seika Kaisha, Ltd.; hereafter referred to as MEEK) and vancomycin hydrochloride 0.5 g for intravenous infusion (Shionogi & Co., Ltd.; hereafter referred to as S-VCM) were used in this study. The VCM drug substance in MEEK (M-VCM) was purchased from Alpharma. In addition, a mixture was prepared by adding D-mannitol (Towakasei Co., Ltd.) and PEG400 (Maruishi Pharmaceutical Co., Ltd.) to S-VCM to obtain the same composition as MEEK (S-VCMPM).

Laboratory animals: Male SD rats (6 weeks old) were purchased from Charles River Japan, Inc. and were used at the age of 7 weeks (body weight: 231 – 265 g) after an acclimatization period of approximately 1 week. During acclimatization, the animals were allowed free access to food and water. The animals were fed a pellet diet for laboratory animals (Oriental Yeast Co., Ltd.), and they were housed and handled according to the “Principles of Laboratory Animal Care”
(NIH publication #85-23, revised 1985) and the “Guide for the Care and Use of Laboratory Animals” (Pharmaceutical Development Department, Meiji Seika Kaisya, Ltd.).

**Preparation of dosing solutions:** Dosing solutions were prepared at concentrations of 4 and 40 mg/mL by reconstituting the test substances in saline JP (Otsuka Pharmaceutical Co., Ltd.) before use. The solutions of MEEK and S-VCMPM contained inactive ingredients at the same ratio to that of the original MEEK (VCM: D-mannitol: PEG4000 = 5:1:1) irrespective of the dose.

**Nephrotoxicity study:** Animals were given MEEK, M-VCM, S-VCM, and S-VCMPM intravenously at a VCM dose of 400 mg/kg in a volume of 10 mL/kg with an infusion rate of 2 mL/minute. The dosing volume was calculated from the body weight measured immediately before administration. The control group was given saline.

Blood urea nitrogen (BUN) was measured 24 hours after dosing. Lactate dehydrogenase (LDH) and \( \alpha \)-N-acetyl-D-glucosaminidase (NAG) were measured in urine samples pooled from immediately to 24 hours after dosing, and total urinary LDH (T-LDH) and total NAG (T-NAG) levels were determined.

After the completion of blood sampling, the animals were immediately exsanguinated by cutting the axillary artery and vein. The kidneys were observed macroscopically, and then were resected. After removal, the kidneys were weighed, fixed in 10% neutral buffered formalin, and sections were cut and stained with hematoxylin and eosin for light microscopy according to conventional procedures.

**Pharmacokinetic study:** After weighing each animal, a polyethylene tube (PE50, Becton Dickinson, and Company) was inserted into the femoral artery for blood collection under ether anesthesia, and a syringe was sutured to the external urethral meatus for collection of urine samples. Then the animal was immobilized in a Bollman cage. After the animal had regained consciousness, dosing solutions of MEEK and S-VCM were administered once at a VCM dose of 40 mg/kg or 400 mg/kg via the tail vein. In the first part of the study, the pharmacokinetics of VCM were examined after administration of MEEK and S-VCM. In the second part of the study, pharmacokinetics were assessed after administration of S-VCM and S-VCMPM to confirm the influence of the inactive ingredients on VCM. Blood samples (200–300 µL) were collected from the 40 mg/kg group at 5, 15, and 30 minutes and 1, 2, 4, 6, and 24 hours after dosing, as well as from the 400 mg/kg group at 5, 15, and 30 minutes and 1, 2, 4, 6, and 24 hours after dosing. The blood was centrifuged (1700 × g for 10 min) with cooling to separate plasma. In the 40 mg/kg group, urine samples were collected immediately after dosing to 2 hours, from 2 to 4 hours, and from 4 to 6 hours. In the 400 mg/kg group, samples were collected at the same times and also from 6 to 24 hours after dosing. Urine volume was determined from the weight using a specific gravity of 1. Plasma and urine samples were stored frozen at –20°C or lower until the measurement of drug concentrations.

**Pharmacokinetics analysis:** The time course of plasma VCM concentrations was analyzed by a model-independent method using WinNonlin (Scientific Consulting, Inc.), and the area under the plasma concentration-time curve (AUC<sub>0-24</sub> and AUC<sub>0-∞</sub>), terminal half-life (T<sub>1/2</sub>), total body clearance (CL<sub>T</sub>), and steady-state volume of distribution (V<sub>ss</sub>) were determined. Renal clearance (CL<sub>R</sub>), nonrenal clearance (CL<sub>N</sub>), and clearance of the unbound drug by tubular secretion (CL<sub>T</sub>) were calculated using the following equations: f: fraction of unbound drug, 0.78); \( \text{CL}_T = \frac{X_{tot}}{AUC_{0-24}} \) (X: total urinary excretion)

\[ \text{CL}_{tot} = \text{CL}_{un} - \text{CL}_r \]

\[ \text{CL}_r = \text{CL}_t - f \cdot \text{GFR} \]

GFR (L/h/kg) = \( \frac{5.92 \times W^{0.77} \times 60}{1000 \times W} \)

(GFR: glomerular filtration rate, W: body weight in kg)

**Statistical analysis:** The significance of differences between the mean pharmacokinetic parameters for the two preparations was examined by Student’s t-test (significance level: P<0.05) using StatView J software (Abacus Concepts, Inc.).

**Results**

**Nephrotoxicity:** The BUN, urinary T-LDH, and urinary T-NAG levels after intravenous administration of the test substances to rats at a VCM dose of 400 mg/kg are shown in Fig. 1 and Fig. 2. The BUN and T-LDH levels in the M-VCM group (19.2 mg/dL, 1931 mIU) and the S-VCM group (24.1 mg/dL, 3516 mIU) were significantly higher than those in the control group (12.4 mg/dL, 182 mIU), while T-NAG tended to be higher than in the control group. In the MEEK group (14.9 mg/dL, 1285 mIU) and the S-VCMPM group (14.2 mg/dL, 1064 mIU), however, BUN and T-NAG values were similar to these in the control group and urinary T-LDH tended to be lower than in the M-VCM or S-VCM groups. Discoloration and a granular surface
of the kidneys were seen in the M-VCM group at autopsy, while the kidneys also showed a granular surface in the S-VCM group. The main histologic findings in the M-VCM and S-VCM groups were tubular epithelial cell degeneration and necrosis, tubular dilatation, urinary casts, and basophilic coloration of the tubular epithelial cells. Hypertrrophy of the glomeruli and dilatation of Bowman’s capsule with retention of eosinophilic material inside Bowman’s space were observed sporadically. On the other hand, most of the animals from the MEEK group and the S-VCM group showed no histological changes. Glomeruli and tubules from rat kidneys are shown in HE-stained sections in Fig. 3 and 4.

**Pharmacokinetics:** The mean plasma concentration vs. time curves following intravenous administration of MEEK and S-VCM to rats at doses of 40 mg/kg and 400 mg/kg are shown in Fig. 5, while the pharmacokinetic parameters of VCM after administration of the two preparations are summarized in Table 1. After administration at a dose of 40 mg/kg, the plasma concentration profiles of the two preparations were virtually the same and both drugs were below the quantification limit at 6 hours after dosing. Elimination was rapid for both preparations, with a T1/2 of approximately 0.5 hr. The AUC0-∞, CL∞, and V∞ also did not differ significantly between the two preparations.

At a dose of 400 mg/kg, however, the plasma VCM concentration was higher in the S-VCM group than in the MEEK group, and VCM was still detected at 24 hours after dosing in the former group. In contrast, VCM was rapidly eliminated from the plasma and was below the quantification limit at 24 hours after dosing in the MEEK group. The T1/2 of VCM in the S-VCM group (3.8 hr) was significantly longer than that in the MEEK group (0.98 hr). The AUC0-∞ of VCM in the S-VCM group (4683 μg·hr/mL) was approximately five times greater than in the MEEK group (947 μg·hr/mL), while CL∞ for the S-VCM group (0.09 L/hr/kg) was approximately one-fifth of that for the MEEK group (0.44 L/hr/kg). V∞ did not differ significantly between the two preparations.

**Figure 6** shows the cumulative urinary excretion of VCM following administration of MEEK and S-VCM to rats at VCM doses of 40 and 400 mg/kg. At a dose of 40 mg/kg, cumulative urinary excretion up to 6 hours after administration did not differ significantly between the MEEK group (88.1% of the dose) and the S-VCM group (86.6% of the dose). At 400 mg/kg, however, the urinary excretion at 4 hours after administration reached 80% of the dose in the MEEK group versus approximately 30% in the S-VCM group. The cumulative urinary excretion up to 24 hours after dosing was

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**Fig. 1.** BUN levels in rats treated with intravenous M-VCM, MEEK, S-VCM, or S-VCM/PM. (Dose: 400 mg/kg, mean ± S.D., n = 5) *: p < 0.05, significantly different from control.

**Fig. 2.** Urinalysis in rats treated with intravenous M-VCM, MEEK, S-VCM or S-VCM/PM. (Dose: 400 mg/kg, mean ± S.D., n = 5) *: p < 0.05, **: p < 0.01, significantly different from control, ***: p < 0.05, significantly different from S-VCM.
Fig. 3. Glomeruli shown by H-E staining of rat kidneys after intravenous administration of S-VCM or MEEK (Dose: 400 mg/kg) A) Dilatation of Bowman’s space, which contains protein-like material in the lumen and hypertrophy of the glomeruli in an S-VCM-treated rat. B) The kidney is almost normal in the MEEK-treated rat.

Fig. 4. Renal cortical tubules shown by H-E staining of rat kidneys after intravenous administration of M-VCM or MEEK (Dose: 400 mg/kg) A) Dilatation of renal tubules, necrosis, or degeneration of the tubular epithelium, and luminal hyaline casts in an M-VCM-treated rat. B) The kidney is almost normal in the MEEK-treated rat.
also significantly lower in the S-VM group (65.9%) than in the MEEK group (89.4%). CLr was calculated using the AUC and the urine volume. At a dose of 40 mg/kg, CLr did not differ significantly between the MEEK group (0.53 L/hr/kg) and the S-VM group (0.46 L/hr/kg). There was also no difference of CLr.

Renal clearance by tubular secretion was observed for both MEEK (0.15 L/hr/kg) and S-VM (0.08 L/hr/kg). At a dose of 400 mg/kg, however, CLr was significantly different between MEEK (0.39 L/hr/kg) and S-VM (0.06 L/hr/kg). In the MEEK group, CLr was not markedly different from that seen at 40 mg/kg, but it was decreased to between one-sixth and one-eighth of that at 40 mg/kg in the S-VM group. In contrast, CLr did not differ significantly between the two preparations (Table 1).

The cumulative urinary excretion up to 6 hours after administration of MEEK and S-VM at a dose of 40 mg/kg was 6–7 mL, and there was no difference of the urine output for any of the sampling intervals. At a dose of 400 mg/kg, the cumulative urinary excretion up to 24 hours after dosing did not differ significantly between MEEK and S-VM (12–15 mL), but the urine output up to 6 hours after dosing was significantly lower for S-VM (Fig. 7).

Pharmacokinetics of S-VM and S-VCMPM were also examined to confirm the influence of the inactive ingredients on VCM. Figure 8 shows the time course of the mean plasma concentration following intravenous administration of S-VM and S-VCMPM at doses of 400 mg/kg. Table 2 shows the pharmacokinetic parameters of VCM and the BUN level after administration of each preparation. The AUCG of the S-VM group (3353 µg·h/mL) was approximately three times greater than that of the S-VCMPM group (1173 µg·h/mL), while CLr and CL of the S-VCMPM group (0.36 and 0.26 L/hr/kg) were approximately twice the values seen in the S-VM group (0.21 and 0.15 L/hr/kg). In contrast, Vmax did not differ significantly between the two preparations. After administration of S-VM and

![Fig. 5. Plasma concentration of VCM after intravenous administration of MEEK or S-VM to rats. (mean ± S.D., n = 4)](image5)

![Fig. 6. Cumulative urinary excretion rate of VCM after intravenous administration of MEEK or S-VM to rats. (mean ± S.D., n = 4)](image6)

| Table 1. Pharmacokinetic parameters of VCM after intravenous administration of MEEK or S-VM to rats. (mean ± S.D., n = 4) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Dose            | 40 mg/kg        | 400 mg/kg       | 40 mg/kg        | 400 mg/kg       |
|                 | MEEK            | S-VM            | MEEK            | S-VM            |
| T1/2 (h)        | 60 ± 8.9        | 76.2 ± 8.8      | 947 ± 191       | 4683 ± 1028*    |
| AUCG (µg·h/mL)  | 0.49 ± 0.08     | 0.49 ± 0.06     | 0.98 ± 0.28     | 3.38 ± 0.36*    |
| CLtot (L/hr/kg) | 0.60 ± 0.08     | 0.53 ± 0.07     | 0.44 ± 0.09     | 0.99 ± 0.02*    |
| Vdss (L/kg)     | 0.34 ± 0.03     | 0.31 ± 0.03     | 0.44 ± 0.04     | 0.39 ± 0.07     |
| CLr (L/hr/kg)   | 0.53 ± 0.10     | 0.46 ± 0.05     | 0.39 ± 0.09     | 0.06 ± 0.02*    |
| CLmr (L/hr/kg)  | 0.07 ± 0.04     | 0.07 ± 0.09     | 0.05 ± 0.02     | 0.03 ± 0.00     |
| CLx (L/hr/kg)   | 0.15 ± 0.10     | 0.08 ± 0.04     | —               | —               |
| f-GFR*          | 0.38 ± 0.003    | 0.38 ± 0.002    | —               | —               |

*: Significant difference from MEEK (400 mg/kg), (p<0.05)

†: f-GFR = 5.92 W-0.78 (body weight, kg0.78)
S-VCPMP, BUN was 45.4 and 22.4 mg/dL, respectively.

**Discussion**

MEEK is a generic drug that is more soluble and stable than the brand-name form of VCM due to the addition of inactive ingredients (D-mannitol and PEG400). As part of the development of this new formulation, nephrotoxicity compared between MEEK and brand-name VCM, and the pharmacokinetics of the two formulations were analyzed.

Following administration of VCM to rats at a nephrotoxic dose (400 mg/kg), there was an increase of BUN indicating renal dysfunction and an increase of urinary T-LDH indicating cell degeneration and necrosis in animals given the drug substances (M-VCM and S-VCM). Histologic examination also revealed renal damage in these animals, including tubular dilatation, tubular epithelial cell degeneration and necrosis (focal), and casts in the urine. It was concluded from these findings that the renal damage caused by VCM extended from the glomeruli to the renal tubules. In the groups treated with VCM formulations that also contained inactive ingredients (MEEK and S-VCPMP), the increases of BUN and urinary T-LDH were suppressed and there were no obvious histologic changes in most of the animals. These results suggested that the inactive ingredients in the MEEK and S-VCPMP preparations could reduce the nephrotoxicity of VCM.

Therefore, we compared the pharmacokinetics of VCM after administration of MEEK and S-VCM. At the nephrotoxic dose of S-VCM, which only contained

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<thead>
<tr>
<th>Parameter</th>
<th>S-VCM</th>
<th>S-VCPMP</th>
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<tr>
<td>T_{1/2} (h)</td>
<td>3.34 ± 1.83</td>
<td>1.86 ± 1.20*</td>
</tr>
<tr>
<td>AUC_{0 → ∞} (mg·h/mL)</td>
<td>3353 ± 2015</td>
<td>1373 ± 298*</td>
</tr>
<tr>
<td>CLTot (L/h/kg)</td>
<td>0.21 ± 0.02</td>
<td>0.36 ± 0.10*</td>
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<tr>
<td>VDes (L/kg)</td>
<td>0.52 ± 0.11</td>
<td>0.61 ± 0.09</td>
</tr>
<tr>
<td>CLR (L/h/kg)</td>
<td>0.15 ± 0.04</td>
<td>0.26 ± 0.08*</td>
</tr>
<tr>
<td>CLnr (L/h/kg)</td>
<td>0.05 ± 0.02</td>
<td>0.03 ± 0.00</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>45.4 ± 24.5</td>
<td>22.4 ± 4.1*</td>
</tr>
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* p < 0.05 S-VCM 40 mg/kg vs S-VCM 400 mg/kg ** p < 0.05 MEEK 400 mg/kg vs S-VCM 400 mg/kg.
the drug substance, CL, decreased to approximately one-eighth (0.06 L/hr/kg) of that at 40 mg/kg and the blood concentration of VCM was increased (Fig. 5, Table 1). The pharmacokinetic data taken together with the toxicity findings suggested that glomerular filtration and tubular secretion may have been suppressed, since these processes play a role in the renal excretion of VCM. The results also suggested that these effects on pharmacokinetics occurred soon after administration of S-VCM because the plasma concentration followed virtually the same time course as that for MEEK up to 30 minutes after dosing, but the elimination of VCM from the blood became slower thereafter and the urine volume and urinary excretion decreased by 2 hours after dosing. In the case of MEEK, which contained the inactive ingredients, the blood VCM concentration decreased rapidly up to 6 hours after dosing, and there were no marked changes of the urine volume, urinary excretion, or CL, when compared with the values obtained at the clinical dosage.

Furthermore, the pharmacokinetics of S-VCM and S-VCM-P were examined to confirm the effect of the inactive ingredients on VCM itself and BUN was also measured at 24 hours after dosing. In the S-VCM-P group, CL, was not markedly decreased like that in the S-VCM group. These results indicated that the inactive ingredients in MEEK had a role in reducing the nephrotoxicity of VCM. The high BUN level in the S-VCM group (45.4 mg/dl) indicated more severe renal damage (Table 2).

These results suggest that the inactive ingredients in MEEK reduce the nephrotoxicity of VCM and contribute to the maintenance of renal function. In our preliminary study, D-mannitol or PEG400 was coadministered separately with VCM, and the results suggested that each of these inactive ingredients had a protective effect against the nephrotoxicity of VCM, but the influence of each substance remains to be studied in detail (data not shown).

Several authors have investigated the nephrotoxicity of VCM and Nagai et al. gave the following explanation of the mechanism involved. After dosing, VCM moves out of the blood and accumulates in the proximal tubular epithelium where it inhibits reabsorption and secretion by the tubules, resulting in a gradual increase in the osmotic pressure of urine passing through the proximal tubules and subsequent formation of casts in the distal tubules. It therefore seems that the inactive ingredients in MEEK may have prevented renal impairment by inhibiting the accumulation of VCM in the kidneys. It has also been reported that antibiotics such as fosfomycin and imipenem-cilastatin can reduce the nephrotoxicity of VCM by inhibiting its uptake into the kidneys. Studies on the mechanism of nephrotoxicity have usually focused on the role of the renal tubules in the excretion of VCM. Although the mechanism by which the inactive ingredients (D-mannitol and PEG400) block the renal effects of VCM is currently unknown, D-mannitol may play a role in altering the distribution of VCM to the kidneys because it increases renal blood flow and the glomerular filtration rate. Studies on the cytotoxic effect of D-mannitol have also suggested that it may help to inhibit renal impairment caused by VCM.

MEEK is expected to be as effective for antibacterial therapy as the brand-name drug, because our study of the influence of the inactive ingredients in MEEK on the nephrotoxicity of VCM confirmed that these ingredients did not alter the pharmacokinetics when MEEK was administered at the usual clinical dose (40 mg/kg as VCM), and the time course of the plasma VCM concentration was the same after administration of MEEK or S-VCM in healthy adults. Further investigation is needed to determine how this reduction in nephrotoxicity is reflected in the clinical setting.

Acknowledgement: We are grateful to Professor Ken-ichi Inui Ph.D. and Satohiro Masuda Ph.D., Department of Pharmacy, Kyoto University Hospital, for constructive advice for this study.

References


17) Package Insert: VANCOMYCIN for I.V. Infusion 0.5 [MEEK].