Brain microdialysis study of vancomycin in the cerebrospinal fluid after intracerebroventricular administration in mice

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PK of vancomycin after *i.c.v.* injection

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Abstract

Vancomycin (VCM) is an important antibiotic for treating methicillin-resistant *Staphylococcus aureus* (MRSA) infections. To treat bacterial meningitis caused by MRSA, it is necessary to deliver VCM into the meninges, but the rate of VCM translocation through the blood-brain barrier is poor. Additionally, high doses of intravascularly (*i.v.*) administered VCM may cause renal impairments. Thus, VCM is sometimes administered intracerebroventricularly (*i.c.v.*) for clinical treatment. However, information on the VCM pharmacokinetics in cerebrospinal fluid (CSF) after *i.c.v.* administration is lacking. In the present study, we evaluated the VCM pharmacokinetics in the CSF and systemic circulation after *i.c.v.* compared to that after *i.v.* administration, using the brain microdialysis method in mice. VCM administered via *i.c.v.* showed a highly selective distribution in the CSF, without migration to systemic circulation. Moreover, to assess renal impairments after *i.c.v.* administration of VCM, we histologically evaluated damage to the mouse kidney by hematoxylin and eosin staining. No significant morphological change in the kidney was observed in the *i.c.v.* administration group compared to that in the *i.v.* administration group. Our results demonstrate that *i.c.v.* administration of VCM can be partially prevented from entering the systemic circulation to prevent renal impairments caused by VCM.

Introduction

Vancomycin (VCM) is an antibiotic specifically used to treat methicillin-resistant *Staphylococcus aureus* (MRSA) infections. For bacterial meningitis caused by MRSA, it is necessary to deliver VCM into the meninges. In contrast, patients are often administered VCM intravenously (*i.v.*). However, the rate at which VCM translocates into the brain is low, with only 1–5% of VCM delivered into the cerebrospinal fluid (CSF) because of the blood-brain barrier (BBB) (1,2). Additionally, increasing the *i.v.* dosage to improve CSF translocation can increase the risk of renal impairments, a common adverse event associated with VCM (3–6). Therefore, intracerebroventricular (*i.c.v.*) injection of VCM can be used for selective administration into the CSF to avoid the poor translocation through the BBB. It has been reported that *i.c.v.* injection of VCM is safe and effective for treating bacterial meningitis caused by MRSA (1). However, few studies have examined the pharmacokinetics of VCM in the CSF after *i.c.v.* administration.

We have been studying a brain microdialysis method (7) and clarified the time-course brain pharmacokinetics after active delivery (8). In the present study, we hypothesized that microdialysis can be used to evaluate the effectiveness and the relationship between pharmacokinetics and nephrotoxicity of *i.c.v.* injection of VCM. We assessed the time-course pharmacokinetics of VCM in the CSF after *i.c.v.* injection and compared the results to those for *i.v.* injection. To assess the risk of renal impairment, we examined the time-course blood concentration of VCM after *i.c.v.* and *i.v.* injection. Moreover, we evaluated renal impairment after one-week administration of VCM by hematoxylin and eosin (HE) staining.

Material and Methods

Animals

Male ddY mice (27–34 g) (Japan SLC, Shizuoka, Japan) were raised in a breeding room (12-h light-dark cycle; 25 ± 1°C) with free access to feed and water. All experiments were executed under the Regulations of the Animal Care and Use Committee of Nagasaki University.

Drug preparation

VCM hydrochloride (Wako Pure Chemical Industries, Osaka, Japan) was dissolved in artificial cerebrospinal fluid (aCSF) (for *i.c.v.* injection) or saline (for *i.v.* injection) and administered to mice at 30 mg/kg. To evaluate renal impairment after one-week administration, 100 mg/kg of VCM in aCSF (*i.c.v.* injection) or saline (*i.v.* injection) was used.

Brain microdialysis
Brain microdialysis was performed as our previous report (8). After fixation of mice, A-I-4-01 probe (Eicom Corporation, Kyoto, Japan) with a 1-mm and 50-kDa-cutoff regenerated cellulose membrane was inserted into the left lateral ventricle of mice (coordinate: A, –0.3; L, +1.0; H, –3.2 mm from Bregma). After 60-min stabilization, VCM was administered (*i.c.v.* or *i.v.*) at 30 mg/kg. The VCM recovery rate of the probes from standard solution was $0.52 \pm 0.18\%$ (n=10).

Blood sampling and preparation
Blood was mixed with 1.25 U/µL of heparin sodium (Mochida Pharmaceutical, Tokyo, Japan) (1:1, v/v %) and centrifuged at 1000 $\times g$ (4°C). The mixture of 60 µL of plasma (three-fold diluted with saline) and 120 µL of methanol (Kanto Chemical, Tokyo, Japan) were centrifuged at 2000 $\times g$ (4°C). The supernatant was analyzed by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) analysis.

Histological evaluation of mice kidney
VCM was administered (*i.c.v.* or *i.v.*) at 100 mg/kg to mice 7 times at 24-h intervals. Twenty-four hours after the final administration, HE staining was performed on sliced kidneys.

HPLC analysis
The HPLC system consisted as previously reported (8). Detection wavelength was set at 205 nm (10). VCM was separated in a mixture of 50 mM phosphate ammonium buffer (pH 2.2) and acetonitrile (88:12, v/v %) at a flow rate of 0.36 mL/min, using Wakopak Handy-ODS column (Wako Pure Chemical Industries).

Pharmacokinetic parameters and statistical analysis

Pharmacokinetic parameters were calculated by moment analysis. Statistical analysis was executed with JMP® Pro 11.2.0 (SAS Institute, Cary, NC). *P*<0.05 was considered to indicate a significant difference.

Results and Discussion

Time-course VCM concentration in CSF

Figure 1a shows the time-course concentration of VCM in the dialysates after *i.c.v.* and *i.v.* administration (30 mg/kg) (n=3) and pharmacokinetic parameters are shown in Table 1. For *i.c.v.* administration, the peak concentration was increased at the first time point, then decreased over time. In contrast, after *i.v.* administration, VCM was not detected in the CSF at any time points. This result agrees with those of Pfausler et al. (1) and suggests that *i.v.* administration of VCM does not easily migrate into CSF. Additionally, after *i.c.v.* administration, VCM was rapidly eliminated from the CSF. Because VCM has both a time-dependent bactericidal action and a long-lasting effect (11), it may be necessary to retain VCM longer in CSF for the effective treatment of bacterial meningitis by MRSA. We believe that the pharmacokinetic data in this study showed can be useful for the clinical use of *i.c.v.* administration of VCM.

Time-course VCM concentration in plasma

After *i.c.v.* and *i.v.* administration (30 mg/kg), VCM concentration in the plasma was quantitated at 6 time points (n=3) (Figure 1b). For *i.v.* administration, the peak concentration was observed at the first point (5 min), after which the concentration decreased over time. In contrast, for *i.c.v.* administration, the VCM concentration remained low and constant. Moreover, the VCM concentration in the CSF decreased rapidly after *i.c.v.* administration (Figure 1a). These results suggest that migration from the CSF to systemic circulation is poor and VCM is eliminated from the CSF. This may be because the inner wall of the cerebral ventricle is composed of ependymal cells, which do not always form tight junctions with other cells (12). Therefore, VCM administered into the CSF may translocate to the brain parenchyma. Additionally, the VCM pharmacokinetics in CSF may be explained by bulk flow, which is the flow of CSF from the brain to the spinal cord (13,14). Thus, VCM administered into the CSF

may have translocated to the brain parenchyma and/or spinal cord, not the systemic circulation.

Renal impairments after i.c.v. *or* i.v. *administration of VCM*

Renal impairments were histologically evaluated by HE staining of the mouse kidneys after one-week VCM administration (100 mg/kg) (Figure 3). No significant morphological change in the kidney was observed between the control group and *i.c.v.* administration group. In contrast, in the *i.v.* administration group, renal tubular dilation and cellular infiltration into the interstitium were observed. To confirm these results, the VCM concentration in the plasma over time after *i.c.v.* and *i.v.* administration (100 mg/kg) was evaluated at 6 time points (n=3) (Figure 2). The time-course concentration (Figure 2) showed good agreement with the HE staining results (Figure 3). Additionally, the result of HE staining after *i.v.* administration of VCM agreed well with those of a study evaluating nephrotoxicity when VCM was repeatedly administered for 3–14 days (15). Therefore, this result suggests that *i.c.v.* administration of VCM does not cause renal impairments in the same dose that nephrotoxicity appeared after *i.v.* administration. Thus, when VCM is administered *i.c.v.*, the dose can be increased without causing adverse effects. VCM administered via *i.c.v.* can be effective for treating bacterial meningitis caused by MRSA.

Conclusion

In this study, we clarified the pharmacokinetics of VCM in the CSF after *i.c.v.* administration, using microdialysis. VCM administered via *i.c.v.* showed a highly selective distribution in the CSF, without migration to systemic circulation and renal impairments.

Acknowledgements

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Legend to Figures

Figure 1 (a) Time-course vancomycin (VCM) concentration in the dialysates from the mouse brain after intracerebroventricular (*i.c.v.*) and intravenous (*i.v.*) administration (30 mg/kg). The VCM concentrations in dialysates after *i.v.* administration were below the limit of quantitation at all time points. (b) Time-course VCM concentration in the mouse plasma after *i.c.v.* and *i.v.* administration (30 mg/kg). (a, b) Data are expressed as the mean ± standard deviation (n=3). *$P<0.05$, **$P<0.01$, and ***$P<0.001$ by Student's *t*-test. N.D., not detected.

Figure 2 Time-course VCM concentration in the mouse plasma after *i.c.v.* and *i.v.* administration (100 mg/kg). (a–c) Data are expressed as the mean ± standard deviation (n=3). *$P<0.05$ and **$P<0.01$ by Student's *t*-test.

Figure 3 Histological assessment of mouse kidneys with hematoxylin and eosin (HE) staining. Doses for *i.c.v.* and *i.v.* administration were 100 mg/kg with one-week administration. Slides from both the cortex and medulla are shown for each group. Scale bar: 50 μm.

Parameter	Dialysate (30 mg/kg)		Plasma (30 mg/kg)		Plasma (100 mg/kg)	
	i.c.v.	*i.v.*	*i.c.v.*	*i.v.*	*i.c.v.*	*i.v.*
C_{max}, µg/mL	12.23 ± 6.21	N.C.	4.92 ± 2.10	74.19 ± 34.11*	5.44 ± 1.28	83.35 ± 20.18**
AUC, µg·min/mL	643.60 ± 242.63	N.C.	469.6 ± 140.9	3158 ± 644.5**	723.7 ± 128.1	6118 ± 643.1***
MRT, min	57.88 ± 13.82	N.C.	88.00 ± 4.47	62.84 ± 9.04*	93.42 ± 3.69	71.86 ± 14.77
$t_{1/2}$, min	43.48 ± 8.72	N.C.	420.9 ± 125.1	97.25 ± 39.84*	1360 ± 1534	198.2 ± 124.8

Table 1 Pharmacokinetic parameters in dialysates from the mouse brain and mouse plasma after intracerebroventricular (*i.c.v.*) and intravenous (*i.v.*) administration of vancomycin (VCM) (30 or 100 mg/kg). The parameters in the dialysate after *i.v.* administration was not calculated because the VCM concentration was below the limit of quantitation. Data are expressed as the mean ± standard deviation (n=3). *P* values were calculated by Student's *t*-test between the *i.c.v.* and *i.v.* groups. *$P<0.05$, **$P<0.01$, and ***$P<0.001$. AUC, area under the curve; MRT, mean residence time; N.C., not calculated.

Figure 1

Figure 2

Figure 3