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Analysis of Vancomycin Blood Concentrations in Critically Ill Patients and Effects of Age, Gender and the *ABCB1* Mutation on Blood Concentrations

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Abstract

Objective: To investigate the clinical application of vancomycin plasma concentration monitoring in critically ill patients and the influence of age, gender and the *ABCB1* gene mutation on vancomycin drug concentrations to guide clinically rational vancomycin usage.

Methods: The vancomycin concentration monitoring data on critically ill patients in our hospital from November 2016 to March 2017 were analyzed. Sixty-eight patients who received the same dosages of vancomycin were subjected to *ABCB1* genotyping.

Results: Among the 141 critically ill patients, 68 (48.22%) showed sub-target concentrations of vancomycin, averaging 5.58 $\mu g.ml^{-1}$ ± 2.54 $\mu g.ml^{-1}$; 29 patients (20.56%) had higher than target concentrations, with an average value of 33.01 $\mu g.ml^{-1}$ ± 9.38 $\mu g.ml^{-1}$; and 44 cases (31.21%) were in the normal concentration range, with an average of 14.72 $\mu g.ml^{-1}$ ± 2.75 $\mu g.ml^{-1}$. Ninety-eight male patients had average vancomycin concentrations of 13.14 $\mu g.ml^{-1}$; 43 female patients had average vancomycin concentrations of 16.19 $\mu g.ml^{-1}$. Vancomycin concentrations in critically ill patients aged 60 years or older were significantly higher than those in patients younger than 60 years (19.71 $\mu g.ml^{-1}$ ± 13.35 $\mu g.ml^{-1}$ *vs.* 10.57 $\mu g.ml^{-1}$ ± 6.67 $\mu g.ml^{-1}$); among the over-60-year-old patients, women had significantly higher concentrations than men (25.4 $\mu g.ml^{-1}$ ± 14.89 $\mu g.ml^{-1}$ *vs.*17.53 $\mu g.ml^{-1}$ ± 12.21 $\mu g.ml^{-1}$). No significant difference in concentration was found between male and female patients younger than 60 years. Genotyping was performed on 68 patients treated with the same vancomycin dosage. The results showed that the vancomycin concentrations in 1236TT-genotype patients were significantly higher than those of the 1236CC and 1236CT-genotype patients. The concentrations in the 2677AA-genotype patients were significantly higher than those in the 2677AT, 2677CA, 2677CC and 2677CT patients. Vancomycin concentrations in 3435TT patients were significantly higher than those in 3435CC, but slightly lower than those in 3435CT patients.

Conclusion: Age and gender may greatly influence vancomycin concentrations in severely ill patients. The *ABCB1* 1236C>*T*, 2677C>*T*/A and 3435C>*T* gene mutations may affect vancomycin concentrations.

Keywords: Vancomycin; Drug concentration monitor; Age; Gender; *ABCB1*

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Introduction

As the first glycopeptide antibiotic, vancomycin is a bactericidal agent with a triple sterilizing mechanism that inhibits bacterial cell wall synthesis, alters bacterial cell membrane permeability, and prevents RNA synthesis in the bacterial cytoplasm. Vancomycin is currently a preferred drug for clinically treating MRSA infections [1,2]; however, its therapeutic window is narrow (the effective and toxic concentrations are similar), and its pharmacokinetics vary largely among patients. Nephrotoxicity (acute renal insufficiency), ototoxicity (ear injury), liver injury, hematological toxicity and other adverse reactions may occur when patients are administered this drug. Therefore, the indications for its clinical application must be strictly understood and the dosing regimen adjusted to the patient's pathophysiology, to keep the blood-drug concentration in a safe range [3]. Many studies have shown that blood-drug concentration monitoring (TDM) can reduce

the potential nephrotoxicity and ototoxicity of vancomycin [4]; therefore, TDM is usually required during vancomycin treatment. Adjusting the dosage based on blood-drug concentration results will help improve vancomycin clinical efficacy and greatly reduce the incidence of adverse reactions. This study analyzed the plasma concentration data on 141 critically ill patients in our department and explored the influence of age, gender and *ABCB1* gene mutations on drug concentrations to provide valuable information for better individualized treatment.

Materials and Methods

Subjects, data source and analysis methods

Critically ill patients who were hospitalized and intravenously administered vancomycin in our hospital from November 2016 to March 2017 were chosen as study subjects. Blood samples were collected for *ABCB1* genotyping from patients who received the same vancomycin dosages and whose blood samples were used in vancomycin blood concentration monitoring. The Hospital Information System (HIS) was used to analyze gender, age, and drug use and vancomycin blood concentration monitoring data. The study was approved by Ethics Committee of hospital.

Blood concentration monitoring

The vancomycin blood concentration was rapidly determined by HPLC. The Agilent HC-C18 column chromatography conditions (4.6 mm × 150 mm, 5 μm) were mobile phase: Methanol/water (containing 0.05 mol/L potassium dihydrogen phosphate, pH=3.5) 20/80 (v/v); detection wave length: 238 nm; flow rate: 1.0 mL/min; column temperature: 35°C; and sample volume: 20 μL. Plasma sample treatment: 600 μL 10% perchloric acid was added to the 200 μL plasma sample, whirlpool and shaking 2 min, 16000 RPM centrifuge 10 min, then 20 μL supernatant was detected by HPLC.

ABCB1 genotyping

Sixty-eight blood samples were collected from critically ill patients, and DNA was extracted and sequenced. The primers were as follows: 1236C>T: Forward 5′-AGCTCTCCACAAAATATCACTAAAAG-3′; Reverse 5′-CAATACAGGTTCCTGAC TCACCACAC-3′; 2677G>T/A: Forward 5′-GTCTGGACAAGCACTGAAAGA -3′; Reverse 5′-GTGGGGAGGAAGGAAGAACA-3′; 3435C>T: Forward 5′-GATCTGTGAACTCTTGTTTTCA-3′; Reverse 5′-GAAGAGAGACTTACATTAGGC-3′. The genotypes were determined from the sequencing results.

Evaluation and study design

Per domestic and international consensus guidelines 5, the target trough concentration of vancomycin was 10 μg.ml^{-1} to 20 μg.ml^{-1} and the trough concentration ranges were divided into 3 levels: <10 μg.ml^{-1}, 10 μg.ml^{-1} to 20 μg.ml^{-1} and >20 μg.ml^{-1}. Patients were grouped by gender to explore the vancomycin concentrations between genders. Simultaneously, vancomycin concentrations were analysed in patients <60 years old and ≥ 60 years old. Genotyping was performed on 68 patients treated with the same dosage of vancomycin. 1236C>T, 2677C>T/A and 3435C>T mutations were analyzed to study the *ABCB1* gene mutation's effect on vancomycin drug concentrations.

Statistical method

SPSS 16 statistical software was used to analyze the data. The measurement data were expressed as X+s, using a t-test. A difference of P<0.05 was considered statistically significant.

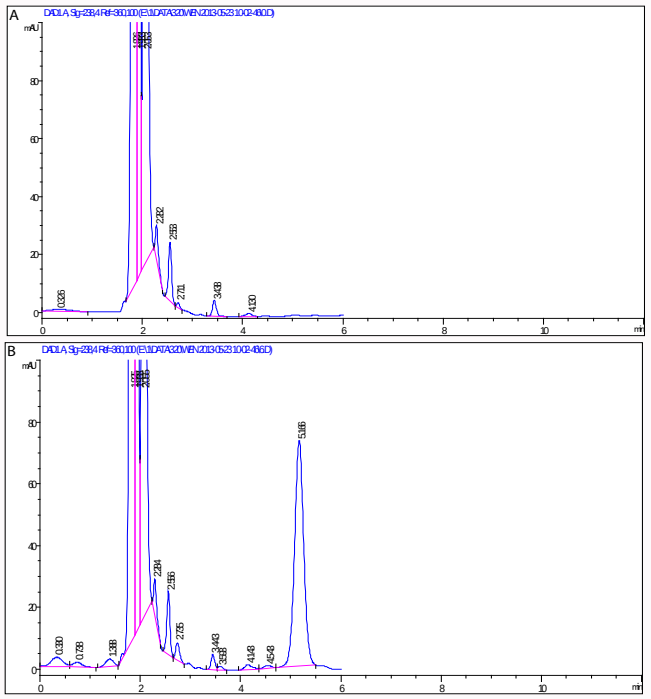

Figure 1: HPLC chromatograms of vancomycin. Blank plasma (A: Blank plasma spiked with vancomycin (64 mg.L^{-1}) (B: The chromatograms peak retention time of vancomycin was at 5.18 min).

Results

Rapid determination of plasma concentration of vancomycin by HPLC

We successfully established a HPLC method for the determination of plasma concentration of vancomycin. The accuracy rate of vancomycin in low, medium and high concentrations (2.0 mg.L^{-1}, 16.0 mg.L^{-1}, 64.0 mg.L^{-1}) were all between 98% to 110%. The inter-day and intra-day Relative Standard Deviations (RSD) were less than 5% (showed in Table 1, n=5). The lower limits of quantification of plasma vancomycin were 2.0 mg.L^{-1}. The linear range was 2.0 mg/L to 64.0 mg/L. The regress equations was y=13.871x+8.8127 (R2=0.9996, p<0.05, n=7). The HPLC chromatogram of vancomycin was showed at Figure 1.

Effect of the *ABCB1* gene mutation on vancomycin concentrations

As shown in the Table 2 and Figure 2, vancomycin concentrations in patients with the 1236TT genotype were significantly higher than those of 1236CC and 1236CT-genotype patients; concentrations in patients with the 2677AA genotype were significantly higher than those of 2677AT, 2677CA, 2677CC and 2677CT patients; and concentrations in patients with the 3435TT genotype were significantly higher than those of 3435CC patients, but slightly lower than those of 3435CT patients.

Analyzing vancomycin target concentrations

Among the 141 critically ill patients, 68 (48.22%) had sub-target concentrations, averaging 5.58 μg.ml^{-1} ± 2.54 μg.ml^{-1}; 29 patients (20.56%) had higher than target concentrations, averaging 33. 01 μg.ml^{-1} ± 9.38 μg.ml^{-1}; and 44 cases (31.21%) were in the normal concentration range, with an average of 14.72 μg.ml^{-1} ± 2.75 μg.ml^{-1}. The total vancomycin mean for all 141 critically ill patients was 14.07 ± 11.54. These results are shown in Table 3 and Figure 3.

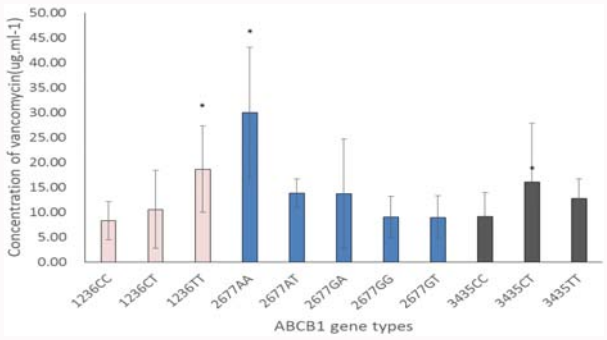

Figure 2: Effect of the *ABCB1* gene mutation on the drug concentration of vancomycin. Vancomycin concentrations in 1236TT-genotype patients were significantly higher than those of the 1236CC and 1236CT-genotype patients. The concentrations in the 2677AA-genotype patients were significantly higher than those in the 2677AT, 2677GA, 2677GG and 2677GT patients. Vancomycin concentrations in 3435CT patients were significantly higher than those in 3435CC, but slightly lower than those in 3435TT patients. *Means significantly difference ($p<0.05$).

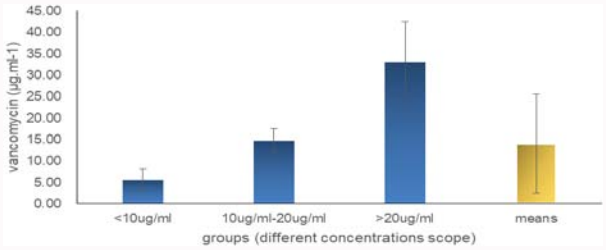

Figure 3: Among the 68 critically ill patients, 31 (45.59%) had sub-target concentrations, averaging 6.40 $\mu g.ml^{-1} \pm 3.76\ \mu g.ml^{-1}$; 17 patients (25.00%) had higher than target concentrations, averaging 26.51 $\mu g.ml^{-1} \pm 8.34\ \mu g.ml^{-1}$; and 20 patients (29.41%) were in the normal concentration range, with an average of 14.72 $\mu g.ml^{-1} \pm 2.75\ \mu g.ml^{-1}$. The total vancomycin mean for all 68 critically ill patients was 13.55 $\mu g.ml^{-1} \pm 5.87\ \mu g.ml^{-1}$.

Vancomycin concentrations between male and female patients

In 141 cases, 98 male patients had a mean vancomycin concentration of 13.14 $\mu g.ml^{-1} \pm 10.55\ \mu g.ml^{-1}$ and 43 female patients had a mean vancomycin concentration of 16.19 $\mu g.ml^{-1} \pm 13.43\ \mu g.ml^{-1}$. These results show that mean vancomycin concentrations in female patients may be higher than those in male patients. These results are shown in Table 4.

Effect of age on vancomycin concentration

The vancomycin concentrations in ≥ 60-year-old critically ill patients were significantly higher than those in patients under 60 years old (19.71 $\mu g.ml^{-1} \pm 13.35\ \mu g.ml^{-1}$ *vs.* 10.57 $\mu g.ml^{-1} \pm 6.67\ \mu g.ml^{-1}$). Female patients ≥ 60 years old had significantly higher concentrations than did male patients in the same age group (25.4 $\mu g.ml^{-1} \pm 14.89\ \mu g.ml^{-1}$ *vs.* 17.53 $\mu g.ml^{-1} \pm 12.21\ \mu g.ml^{-1}$), while no significant differences in concentrations were seen between men and women 60 years old (10.25 $\mu g.ml^{-1} \pm 8.19\ \mu g.ml^{-1}$ *vs.* 11.26 $\mu g.ml^{-1} \pm 9.67\ \mu g.ml^{-1}$). These results are shown in detail in Tables 5 and 6.

Discussion

The most common adverse reactions to vancomycin include fever, chills, and phlebitis [5]. Red man syndrome may be associated with histamine release, manifested as tingling and facial, neck, and upper body flushing, and usually occurs with a large-dose rapid infusion of vancomycin (>500 mg, infusion within 30 min) [6]. Vancomycin requires at least 1 h of infusion to reduce adverse reactions. For higher concentrations (2 g), infusion time should be more than 1.5 h to 2 h. Nephrotoxicity and ototoxicity are serious adverse effects of vancomycin, which are closely related to high plasma concentrations of vancomycin; therefore, vancomycin concentrations must be closely monitored to reduce toxicity. A meta-analysis showed that TDM significantly improved the clinical efficacy of vancomycin treatment and reduced the incidence of nephrotoxicity [7]; therefore, plasma concentrations of vancomycin must be detected.

We used HPLC technology to rapidly and efficiently detect

Table 1: Inter-day and intra-day precision and accuracy of vancomycin.

Added (μg/ml)		Measured (μg/ml)					Mean	SD	RSD (%)	RE (%)
		1	2	3	4	5				
Batch 1	4	3.87	4.61	4.36	3.79	4.01	4.13	0.35	8.4	3.2
	16	16.92	16.63	16.42	16.3	16	16.46	0.34	2.05	2.86
	64	67.23	66.56	64.6	64.9	63.7	65.38	1.47	2.25	2.16
Batch 2	4	4.02	4.04	4.08	4.08	4.12	4.07	0.04	0.96	1.7
	16	17.61	16.56	16.68	14.8	15.7	16.27	1.07	6.57	1.66
	64	60.04	66.19	65.32	59.9	63	62.89	2.92	4.64	-1.73
Batch 3	4	4.32	3.81	4.06	4.54	4.41	4.23	0.29	6.91	5.7
	16	17.47	18.04	17.29	17.9	17.9	17.71	0.32	1.79	10.7
	64	69.93	63.28	67.4	67.5	67.8	67.18	2.41	3.59	4.97

Table 2: Effect of the *ABCB1* gene mutation on vancomycin concentrations.

Gene mutation	1236C>T			2677C>T/A					3435C>T		
Gene types	1236CC	1236CT	1236TT	2677AA	2677AT	2677CA	2677CC	2677CT	3435CC	3435CT	3435TT
	n=10	n=38	n=20	n=10	n=10	n=26	n=12	n=10	n=20	n=38	n=10
CONC											
Means	8.27	10.54	18.65	30.03	13.82	13.72	9.04	8.96	9.09	16.12	12.72
SD	3.82	7.83	8.69	13.07	2.88	11	4.11	4.31	4.9	11.75	3.99

Table 3: Analysis of target concentration range of vancomycin in 141 patients.

Different range		10 μg.ml^{-1}	10 to 20 μg.ml^{-1}	>20 μg.ml^{-1}
	(n=141)	(n=68)	(n=44)	(n=29)
X	14.07	5.58	14.72	33.01
SD	11.54	2.54	2.75	9.38

Table 4: Vancomycin concentrations between genders.

Groups	Total means (n=141)	men (n=98)	women (n=43)
X	14.07	13.14	16.19
SD	11.54	10.55	13.43

Table 5: Average vancomycin concentrations by gender in patients aged >60 years.

Groups	Total means (n=54)	men (n=39)	women (n=15)
X	19.71	17.53	25.4
SD	13.35	12.21	14.89

Table 6: Average vancomycin concentrations by gender in patients aged <60 years.

Groups	Total means (n=87)	men (n=59)	women (n=28)
X	10.57	10.25	11.26
SD	6.67	8.19	9.67

vancomycin concentrations; Methodological evaluation results showed that the method met the requirements of biological sample analysis. Vancomycin penetration in tissues and body fluids is low, and the "China Vancomycin Clinical Application Expert Consensus" indicated that to achieve clinical efficacy, vancomycin troughs should be controlled at concentrations of 10 mg/L to 20 mg/L [6]. We also used this as a standard to detect and judge the effective concentrations of vancomycin. This study showed that in the 141 cases of critically ill patients, 68 (48.22%) were lower than the target concentration range, 29 (20.56%) were higher than the target concentration range, and 44 (31.21%) were within the normal concentration range. Most critically ill patients fail to reach or exceed the effective concentration range after receiving vancomycin, and the reasons for this may be closely related to disease conditions, gender, age, or genetics. We found that both age and gender are important for vancomycin concentrations in patients with severe diseases. The average concentration in female patients was higher than that in male patients. The average concentration in patients older than 60 years was higher than that in patients under 60, and the average concentration in female patients over 60 years of age was significantly higher than that in male patients in that age group; however, the average concentrations in male and female critically ill patients under 60 years old did not significantly differ. Therefore, age and gender have a greater impact on vancomycin concentrations in critically ill patients. In clinical practice, changing non-genetic factors should be considered.

We also studied the *ABCB1* mutation's effect on plasma concentrations of vancomycin. The results showed that the vancomycin concentration in 1236TT-genotype patients was significantly higher than that in patients with the 1236CC and 1236CT genotypes; the vancomycin concentration of 2677AA-genotype patients was significantly higher than that in 2677AT, 2677CA, 2677CC, and 2677CT patients; and 3435TT patient concentrations were significantly higher than those in 3435CC patients, but slightly lower than those in 3435CT patients. However, because only 68 patients were genotyped (patients with the same vancomycin treatment dosage), more cases are needed to support this conclusion. At the same time, whether Vancomycin was the substrate of P-gp was not clear at present.

Patient weight, disease status, diet and combined medications can also alter the body's internal processes, affecting drug absorption, distribution, metabolism, and excretion, ultimately resulting in different drug concentrations and pharmacological effects. But for this study, the influence of age and gender on vancomycin plasma concentrations in the Chinese population is novel and interesting.

Conclusion

In conclusion, this was the first study to investigate the effects of age, gender and *ABCB1* mutations on drug concentrations. The results showed that age and sex greatly influenced vancomycin concentrations in critically ill patients. The *ABCB1* gene mutation affected vancomycin drug concentrations, which were all increased with 1236C>T, 2677C>T/A and 3435C>T mutations, but more subjects are required for further studies.

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References

1. Diaz R, Afreixo V, Ramalheira E, Rodrigues C, Gago B. Evaluation of vancomycin MIC creep in methicillin-resistant Staphylococcus aureus infections- a systematic review and meta-analysis. Clin Microbiol Infect. 2018;24(2):97-104.
2. Ye ZK, Chen YL, Chen K, Zhang XL, Du GH, He B, et al. Therapeutic drug monitoring of vancomycin: A guideline of the Division of Therapeutic Drug Monitoring, Chinese Pharmacological Society. J Antimicrob Chemother. 2016;71(11):3020-5.
3. Martin JH, Norris R, Barras M, Roberts J, Morris R, Doogue M, et al. Therapeutic monitoring of vancomycin in adult patients: A consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. Clin Biochem Rev. 2010;31(1):21-4.
4. Tobler A, Muhlebach S. Intravenous phenytoin: A retrospective analysis of Bayesian forecasting versus conventional dosing in patients. Int J Clin Pharm. 2013;35(5):790-7.
5. Darko W, Medicis JJ, Smith A, Guharoy R, Lehmann DE. Mississippi mud no more: Cost-effectiveness of pharmacokinetic dosage adjustment of vancomycin to prevent nephrotoxicity. Pharmacotherapy. 2003;23(5):643-50.
6. Ye ZK, Li C, Zhai SD. Guidelines for therapeutic drug monitoring of vancomycin: A systematic review. PLoS One. 2014;9(6):e99044.
7. Ye ZK, Tang HL, Zhai SD. Benefits of therapeutic drug monitoring of vancomycin: A systematic review and meta-analysis. Plos One. 2013;8(10):e77169.