Increased vancomycin dosing requirements in sickle cell disease due to hyperfiltration-dependent and independent pathways

Glomerular hyperfiltration is common among patients with sickle cell disease (SCD), likely driven by increased renal blood flow and glomerular hyperperfusion.[1] In other conditions, hyperfiltration is associated with increased renal clearance of drugs, especially antibiotics,[2] but evidence in SCD patients is lacking. Vancomycin, an antibiotic primarily excreted *via* glomerular filtration, is commonly used to treat gram-positive pathogens. The drug level is frequently measured to optimize the dosing regimen.[3] We studied vancomycin as a model to show that patients with SCD had increasing drug dosing requirements. Pathway analysis and gene profiling analysis demonstrated that both hyperfiltration-dependent and -independent mechanisms appear to contribute to increased drug clearance in this patient population.

Using a pharmacy order reporting system, we conducted a retrospective cohort study of SCD adults with a creatinine clearance (CrCl) of 80 mL/min or more, as calculated by the Cockcroft-Gault equation, who were treated with intravenous vancomycin in an academic medical center from 2011 to 2015 and had a vancomycin trough level drawn at steady state after at least three doses. A control group of age- (± 5 years), gender-, weight- (± 10%), and race-matched non-SCD patients with CrCl of 80 mL/min or more were identified using the same pharmacy order reporting method. CrCl of 80 mL/min or more is a commonly used cut off for standard *versus* renal-adjusted vancomycin dosing regimens. The SCD patients excluded for a CrCl less than 80 mL/min accounted for approximately 10% of the patients meeting the other inclusion criteria. Vancomycin doses, trough level at steady state, and other clinical variables were collected through retrospective chart review. The study was approved by the Institutional Review Board.

We identified 104 unique patients meeting the inclusion criteria in the SCD and control groups, providing a study population of 208. Ninety-two percent of the patients (n=96) in the SCD group were hemoglobin SS (HgbSS) genotype and 8% were HgbSC genotype. HgbSS and HgbSC genotypes were compared to their respective HgbAA controls (Table 1). In the HgbSS *versus* HgbAA comparison, median age was 29 and 30 years, respectively, and 53% were males in both groups (Table 1). Patient weight was also comparable (69 kg *vs.* 70 kg). In 76% of the HgbSS patients, the indication for vancomycin was acute chest syndrome (ACS). The most common indications for vancomycin treatment in the control group included fever (21%), sepsis (13%), pneumonia (11%), abscess (9%), cellulitis (7%), wound infection (7%), and meningitis (5%). For the majority of the indications in both groups, the target vancomycin trough level was 15-20 mg/L.[4] The HgbSS patients had higher CrCl and eGFR, as estimated using the Chronic Kidney Disease Epidemiology Collaboration formula,[5] compared to the HgbAA group (Table 1). The first vancomycin trough level at steady state was comparable between the two groups (8.7 *vs.* 8.8 mg/L). The median vancomycin dose and weight-based dose at the trough level were approximately 20% higher in the HgbSS patients (Table 1). The incidence of acute kidney injury was comparable between the HgbSS and HgbAA groups. The HgbSC and matched controls had 8 subjects in each group, and the comparisons failed to show statistical significance, likely due to the small numbers of subjects.

A linear regression analysis of the vancomycin trough level was performed in a multivariate analysis that adjusted for age, gender, SCD genotype (HgbAA, HgbSC, and HgbSS coded as 0, 1, and 2), and weight-based vancomycin dose. SCD genotype was an independent correlate of lower trough level (β = -0.083, 95%CI: -0.023 to -0.144; P=0.007) (Figure 1A). We hypothesized that increased renal clearance due to glomerular hyperfiltration could be one of the mechanisms causing the higher dosing requirements in the SCD group. Consistent with our hypothesis, CrCl or eGFR was an independent, significant predictor of the trough level (P<0.001) when added to this model, whereas SCD genotype was less significant (P=0.036 and P=0.112, respectively).

We also performed pathway analysis of vancomycin trough level using structural equation modeling.[6] The fit-

Table 1. Clinical characteristics in the sickle cell disease and non-sickle cell disease groups.

	HgbSS	HgbAA control	*P*	HgbSC	HgbAA control	*P*
N	96	96		8	8	
Age (years)	29 (24-34)	30 (22-34)	0.370	25 (22-37)	23 (21-39)	0.916
Gender (male %)	53	53	NS	38	38	NS
Weight (kg)	69 (59-78)	70 (60-76)	0.671	71 (60-80)	69 (61-77)	0.875
Race (AA %)	97	97	NS	100	100	NS
Length of treatment (days)	5 (3-7)	5 (3-7)	0.794	4 (3-8)	5 (2-9)	0.958
Doses received during treatment	12 (7-18)	10 (7-16)	0.165	11 (7-19)	11 (6-20)	0.916
Baseline SCr (mg/dL)	0.59 (0.52-0.73)	0.69 (0.57-0.82)	0.002	0.67 (0.60-0.76)	0.64 (0.58-0.87)	0.674
Baseline CrCl (mL/min)	137 (114-165)	120 (102-150)	0.018	108 (93-128)	120 (98-141)	0.494
Baseline eGFR (mL/min/1.73 m^2)	149 (138-160)	141 (126-154)	0.002	136 (129-153)	141 (120-150)	0.875
Trough level at steady state (mg/L)	8.7 (6.5-11.5)	8.8 (6.5-12.4)	0.557	9.7 (5.2-13.7)	12.1 (6.9-15.7)	0.599
Daily dose to reach trough level (gram/day)	3 (2.3-3.5)	2.5 (2.0-3.0)	0.013	2.5 (2.3-3.4)	2 (2.0-2.8)	0.165
Daily dose/weight at trough level (gram/day/kg)	0.043 (0.034-0.049)	0.036 (0.032-0.044)	0.003	0.040 (0.033-0.046)	0.034 (0.030-0.038)	0.128
Acute kidney injury (%)	4.2	5.2	0.749	0	12.5	0.273

Median (interquartile range) or percent is shown. Patients' characteristics were compared using Mann-Whitney U test for continuous variables or χ^2 test or Fisher exact test for categorical variables.

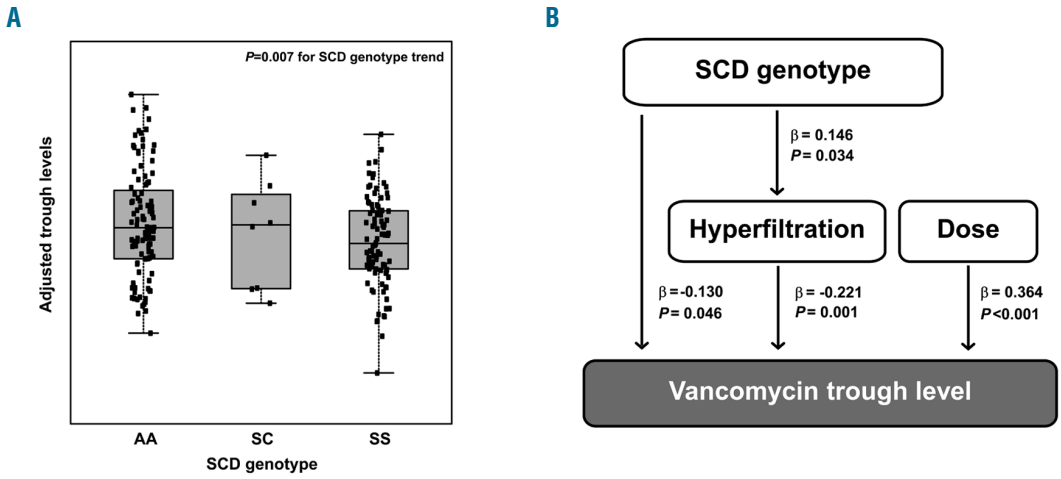

Figure 1. The vancomycin trough level in different sickle cell disease (SCD) genotypes. (A) A linear regression analysis was performed of log vancomycin trough level as a function of SCD genotype (HgbAA, HgbSC, HgbSS coded as 0, 1, and 2), log daily vancomycin dose/weight, log age and gender. The trough level on the y-axis is adjusted for age, gender, and vancomycin dose/weight; n=208. (B) Pathway analysis for the vancomycin trough level. Standardized βs and *P*-values are indicated in the model. Comparative fit index (CFI) = 1.000; goodness-of-fit χ^2 test *P*=0.3; Root Mean Square Error of Approximation of model less than 0.001. According to this analysis: i) the SCD genotypes (HgbAA, SC, and SS were coded as 0, 1, and 2) was associated with hyperfiltration; and ii) hyperfiltration was associated with lower vancomycin trough level. Both lower dose of vancomycin and SCD genotype had additional contributions to lower vancomycin trough level independent of hyperfiltration. CrCl more than 165 mL/min was used to define hyperfiltration, and the dose of vancomycin was adjusted by weight.

ness of the model was jointly determined by the goodness-of-fit test, comparative fit index (CFI), and the root mean square error of approximation (RMSEA). A CFI more than 0.9, goodness-of-fit χ^2 test, *P*-value more than 0.05, or RMSEA less than 0.05 were considered to be an excellent fit of the data to the model. Our data best fit a model in which, consecutively: i) the SCD genotype was associated with hyperfiltration; and ii) hyperfiltration was associated with lower vancomycin trough level. In addition, both lower dose of vancomycin and SCD genotype had additional contributions to lower vancomycin trough level, independent of hyperfiltration (Figure 1B) (CFI=1.000, goodness-of-fit χ^2 test *P*=0.3, RMSEA <0.001). There is no commonly agreed definition for hyperfiltration based on a CrCl or eGFR cut off in SCD or other conditions, with thresholds ranging from 125-175 mL/min.[7] When a series of CrCl threshold cut offs for hyperfiltration (100-200 mL/min) were tested in the pathway analysis, 165 mL/min fit the model best.

To explore potential SCD-specific drug metabolism independent of renal clearance, we examined a list of genes involved in drug metabolism (transporters, phase I and II metabolism enzymes) for enrichment in genes differentially expressed at a 1.2-fold change or more at 5% false discovery rate (Table 2). These genes had been identified in a previous study that compared 13 African-American HgbSS patients and 16 African-American HgbAA control individuals, all at steady state.[8] Among the genes interrogated by the Affymatrix Exon array and analyzed previously,[8] genes involved in drug metabolism were enriched by 2.1-fold in the genes differentially expressed in SCD (χ^2 test, *P*=0.0032), suggesting that drug metabolism-related transcription, enriched in hepatocytes, is prone to be altered in SCD.

As SCD is a condition that compromises the immune system, patients are at an increased risk for developing invasive infections warranting antibiotic treatment,[9] and it has been shown that use of standard dosing regimens when treating SCD patients with gentamicin leads to suboptimal drug concentrations.[10] Our results demonstrate that the vancomycin-dosing requirement is increased by approximately 20% (0.043 *vs.* 0.036 gram/day/kg) in the HgbSS group to achieve a comparable drug level to HgbAA patients. Most of the vancomycin is excreted unchanged *via* glomerular filtration,[3] and glomerular hyperfiltration has caused increased vancomycin dosing requirements in other disease states.[2] A limitation to our study is that using CrCl as an estimation of kidney function in patients with SCD may over-estimate the GFR due to reduced muscle mass.[11] In the pathway analysis, we found evidence that SCD contributed to lower vancomycin trough levels *via* both hyperfiltration-dependent and -independent mechanisms. The fitness of the pathway model was best when a hyperfiltration threshold was used instead of CrCl as a linear variable, consistent with hyperfiltration as one mechanism for increased drug clearance. SCD patients have increased hepatic and renal blood flow, and both may contribute to increased clearance of morphine in this patient population.[12] It has been shown that 30% of vancomycin clearance is *via* a non-renal mechanism[13] and that the half-life of vancomycin is prolonged in patients with impaired liver function.[14] Although the exact metabolism of vancomycin is still unclear, the non-renal clearance is likely *via* hepatic conjugation based on the availability of functional groups in the vancomycin molecular structure.[15]

In summary, we found that HgbSS patients with normal kidney function had an approximately 20% higher vancomycin dosing requirement to reach comparable drug levels in HgbAA patients. The higher dosing requirement for vancomycin appears to be in part due to glomerular hyperfiltration and in part due to increased drug metabolism. Despite this finding, we cannot make a general recommendation that vancomycin dosing be higher in SCD, anticipating glomerular hyperfiltration, because many SCD patients have chronic kidney disease with low CrCl and others may experience acute kidney injury during vaso-occlusive pain crisis.[1] Our results do call for especially close monitoring of vancomycin levels and doses in patients with SCD, and they underscore the need to further investigate the pharmacokinetics and metabolism of vancomycin and other drugs in this patient population.

Table 2. Differential expressions of genes in drug metabolism pathways.

Direction	Gene	Fold	Gene annotation	Category
Increased	PTGS2	1.94	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	phase I
	AQP9	1.92	aquaporin 9	transporter
	SLC7A5	1.86	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	transporter
	ACSL1	1.56	acyl-CoA synthetase long-chain family member 1	phase II
	SLC2A3	1.36	solute carrier family 2 (facilitated glucose transporter), member 3	transporter
	SLC2A1	1.35	solute carrier family 2 (facilitated glucose transporter), member 1	transporter
	CYP4F3	1.31	cytochrome P450, family 4, subfamily F, polypeptide 3	phase I
	CYP1B1	1.24	cytochrome P450, family 1, subfamily B, polypeptide 1	phase I
	SLC16A1	1.24	solute carrier family 16, member 1 (monocarboxylic acid transporter 1)	transporter
	ABCG2	1.24	ATP-binding cassette, sub-family G (WHITE), member 2	transporter
	SLC16A3	1.24	solute carrier family 16, member 3 (monocarboxylic acid transporter 4)	transporter
	ALDH1A1	1.24	aldehyde dehydrogenase 1 family, member A1	phase I
	PTGS1	1.21	prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	transporter
	SLC3A2	1.2	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	transporter
	MGST1	1.2	microsomal glutathione S-transferase 1	phase II
Decreased	SLC7A6	1.45	solute carrier family 7 (cationic amino acid transporter, y+ system), member 6	transporter
	ABCA5	1.32	ATP-binding cassette, sub-family A (ABC1), member 5	transporter
	GZMA	1.29	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	phase I
	EPHX2	1.28	epoxide hydrolase 2, cytoplasmic	phase II
	SLC15A2	1.25	solute carrier family 15 (H+/peptide transporter), member 2	transporter
	CYP2R1	1.2	cytochrome P450, family 2, subfamily R, polypeptide 1	phase I
	ABCD3	1.2	ATP-binding cassette, sub-family D (ALD), member 3	transporter

The complete list of genes examined were from Qiagen Human Drug Transporters, phase I enzyme and phase II enzyme PCR arrays. (*http://www.sabiosciences.com/rt_pcr_product/HTML/PAHS-070Z.html, http://www.sabiosciences.com/rt_pcr_product/HTML/PAHS-068A.html, http://www.sabiosciences.com/rt_pcr_product/HTML/PAHS-069A.html*).

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