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# Safety, Tolerability, Systemic Exposure, and Metabolism of CRS3123, a Methionyl-tRNA Synthetase Inhibitor Developed for Treatment of *Clostridium difficile*, in a Phase 1 Study

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**ABSTRACT** *Clostridium difficile* causes antibiotic-associated diarrhea and is a major public health concern. Current therapies disrupt the protective intestinal flora, do not reliably prevent recurrent infections, and will be decreasingly effective should less susceptible strains emerge. CRS3123 is an oral agent that inhibits bacterial methionyl-tRNA synthetase and has potent activity against *C. difficile* and aerobic Gram-positive bacteria but little activity against Gram-negative bacteria, including anaerobes. This first-in-human, double-blind, placebo-controlled, dose escalation study evaluated the safety and systemic exposure of CRS3123 after a single oral dose in healthy adults. Five cohorts of eight subjects each received CRS3123 or placebo in a 3:1 ratio. Doses for the respective active arms were 100 mg, 200 mg, 400 mg, 800 mg, and 1,200 mg. Blood and urine were collected for pharmacokinetic analysis. CRS3123 concentrations were measured with validated LC-MS/MS techniques. There were no serious adverse events or immediate allergic reactions during administration of CRS3123. In the CRS3123-treated groups, the most frequent adverse events were decreased hemoglobin, headache, and abnormal urine analysis; all adverse events in the active-treatment groups were mild to moderate, and their frequency did not increase with dose. Although CRS3123 systemic exposure increased at higher doses, the increase was less than dose proportional. The absorbed drug was glucuronidated at reactive amino groups on the molecule, which precluded accurate pharmacokinetic analysis of the parent drug. Overall, CRS3123 was well tolerated over this wide range of doses. This safety profile supports further investigation of CRS3123 as a treatment for *C. difficile* infections. (This study has been registered at ClinicalTrials.gov under identifier NCT01551004.)

**KEYWORDS** CRS3123, *Clostridium difficile*, antimicrobial agents, glucuronidation, Gram-positive bacteria, pharmacokinetics

*Clostridium difficile* infection (CDI) is a toxin-mediated diarrheal disease caused by the anaerobic Gram-positive, spore-forming bacterium *Clostridium difficile* (1). From 2001 to 2010, after the emergence of the hypervirulent strain BI/NAP1/027 (2), the incidence of CDI increased from 4.5 per 1,000 adult hospital discharges to 8.2 per 1,000 discharges (3). CDI is associated with significant morbidity and mortality and over \$1.5 billion in health care-related costs annually in the United States (4).

CDI is now the primary cause of antibiotic-associated colitis and nosocomial

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antibiotic-associated diarrhea (AAD) (5, 6). The initial step in the causation of CDI is ingestion of spores that are spread by the fecal-oral route and that contaminate hospital environments and health care workers (7). *C. difficile* spores are resistant to disinfectants and remain on environmental surfaces for up to 12 months with minimal loss of infectivity (8). They remain in intestinal crypts after planktonic bacteria have been eliminated, germinate after treatment, and cause recrudescence disease.

Metronidazole and vancomycin are recommended for treatment of CDI (9, 10), but neither drug is sporocidal, and treatment failures and recurrences are an increasing clinical challenge (11, 12). Both of these antibiotics also disrupt the normal colonic microbiota, resulting in increased risk of reinfection (11); furthermore, *C. difficile* strains that are resistant to both drugs are emerging at an alarming rate (13). A relatively new antibiotic, fidaxomicin, has a somewhat narrower spectrum of activity and lower recurrence rates, but only for non-BI/NAP1/027 strains (14); it has not been widely used due to cost (15, 16).

The ideal antibiotic for the treatment of CDI would have low oral bioavailability, be effective against planktonic bacteria, prevent sporulation and germination of *C. difficile*, and have little effect on the gut microbiota. CRS3123, a novel, fully synthetic diaryl-diamine, appears to meet these criteria (17, 18). It inhibits the bacterial methionyl-tRNA synthetase (MetRS) of Gram-positive bacteria, including *C. difficile*, but has little effect on the structurally distinct MetRS of most Gram-negative bacteria and mammals, including humans (17). At concentrations as low as 1  $\mu\text{g/ml}$ , CRS3123 inhibited *in vitro* toxin production by stationary-phase organisms at high density and reduced sporulation more than 10-fold (18). At concentrations as low as 0.5 mg/kg of body weight, CRS3123 was effective in the hamster model of CDI and superior to vancomycin in survival at 33 days (18). This suggests that the drug both inhibits sporulation and prevents germination of *C. difficile*.

In *in vitro* studies, CRS3123 was active against a broad range of *C. difficile* strains, including epidemic BI/NAP1/027 strains (MIC, 0.5 to 1  $\mu\text{g/ml}$ ), as well as other clinically important aerobic Gram-positive cocci, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococcus faecalis*, and *Enterococcus faecium* (MIC<sub>90S</sub> < 1  $\mu\text{g/ml}$ ) (17), but was inactive against some of the major intestinal Gram-positive colonizers, including *Lactobacillus* and *Bifidobacterium* as well as all Gram-negative bacteria (19). Selectivity for the MetRS of *C. difficile* was >1,000-fold over human mitochondrial MetRS and >1,000,000-fold over human cytoplasmic MetRS (17).

Animal pharmacology and toxicology studies conducted by intravenous (i.v.) and oral routes of administration in Sprague-Dawley rats and beagle dogs supported progression into clinical testing in human subjects with adequate safety margins. Oral bioavailability of CRS3123 was <1% in hamsters, 1 to 10.5% in rats, and <1 to 7.3% in dogs (Crestone Inc., unpublished data).

These *in vitro* and animal studies supported further development of CRS3123, and we assessed the safety, tolerability, and systemic exposure of escalating doses of CRS3123 in healthy volunteers (ClinicalTrials registration no. NCT01551004).

## RESULTS

**Demographic characteristics.** Forty research participants were enrolled, randomized, and given study product or placebo. All 40 subjects completed the study as planned; there were no subject discontinuations. All subjects who received any study drug were included in the “safety population” and in the “modified intention-to-treat population” for analysis. Twenty-nine of 30 subjects treated with active study medication had adequate blood samples collected for all drug quantitation. One subject in cohort 2 (CRS3123, 200 mg) had a baseline blood sample of insufficient volume; he was not discontinued from further participation.

The 30 subjects given CRS3123 did not differ from the 10 placebo recipients with respect to mean age (26.8 years versus 30.1 years, respectively), race (50.0% white for both), or mean BMI (25.2 kg/m<sup>2</sup> versus 23.9 kg/m<sup>2</sup>, respectively). CRS3123-treated subjects differed from the placebo recipients with respect to gender (male, 17/30

**TABLE 1** Demographics of the study population<sup>a</sup>

Characteristic	Cohort A (100 mg, n = 6)	Cohort B (200 mg, n = 6)	Cohort C (400 mg, n = 6)	Cohort D (800 mg, n = 6)	Cohort E (1,200 mg, n = 6)	Placebo cohort (n = 10)
Male (%)	2 (33.3)	4 (66.7)	4 (66.7)	3 (50.0)	4 (66.7)	9 (90.0)
Mean age (SD)	29.3 (4.8)	23.2 (5.2)	24.5 (6.8)	27.8 (6.2)	29.2 (6.2)	30.1 (7.0)
White (%)	2 (33.3)	4 (66.7)	4 (66.7)	2 (33.3)	3 (50.0)	5 (50.0)
African American (%)	2 (33.3)	2 (33.3)	2 (33.3)	3 (50.0)	3 (50.0)	5 (50.0)
Asian (%)	2 (33.3)	0	0	1 (16.7)	0	3 (7.5)
Mean BMI in kg/m <sup>2</sup> (SD)	26.1 (4.4)	24.0 (3.7)	25.1 (2.3)	25.2 (2.0)	25.7 (4.9)	23.9 (3.6)

<sup>a</sup>Values are numbers (% or SD, as indicated, in parentheses).

[56.7%] versus 9/10 [90%], respectively), and ethnicity (non-Hispanic or non-Latino, 25/30 [83.3%] versus 10/10, respectively). Demographic characteristics are provided in [Table 1](#).

**Safety profile.** Adverse events (AEs) were reported in 93.3% (28/30) of the CRS3123-treated subjects and in 90.0% (9/10) of the placebo-treated subjects. A summary of AEs that occurred in two or more subjects in any of the cohorts is presented in [Table 2](#). There were no serious adverse events or deaths; no acute allergic reactions occurred during administration of the study drug. In the combined CRS3123 cohorts, the AEs with the highest frequency ( $\geq 5\%$ ) were decreased hemoglobin (7 [23.3%]), headache (6 [20.0%]), abnormal urine analysis (6 [20.0%]), and positive urine leukocyte esterase (5 [16.7%]). Among the placebo recipients, the highest-frequency AEs ( $\geq 5\%$ ) were abnormal urine analysis (3 [30.0%]) and headache, nasal congestion, upper respiratory tract infection, blood glucose increase, and myalgia (2 [20%], each). No clinically significant electrocardiogram (ECG) abnormalities were noted.

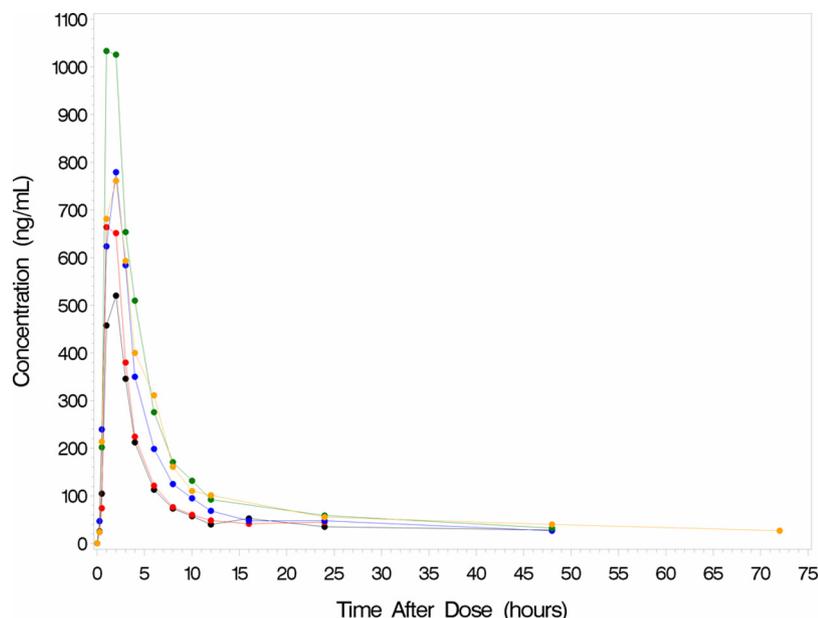
AE frequency in the CRS3123 cohorts did not increase with increasing dose of study drug; the CRS3123 400-mg cohort reported the lowest percentage of AEs (66.7%), whereas the 100 mg, 200 mg, 800 mg, and 1,200 mg cohorts all reported at least one AE among all participants. The majority of AEs were classified as mild (overall, 137/148 [92.6%]). Subjects treated with CRS3123 reported nine moderate AEs, compared with only one reported by the placebo-treated participants. AE severity did not correlate with the dose of CRS3123; the 1,200-mg cohort experienced fewer mild AEs (24 AEs) than the 100-mg and 800-mg cohorts (27 AEs each). There were no severe AEs among the CRS3123-treated subjects. One placebo recipient had a severe AE of proteinuria, which resolved without intervention.

Sixty-five (53.7%) of the AEs reported by CRS3123 recipients were deemed to be associated with the study product, and 13 (48.1%) of those experienced by placebo recipients were considered associated. The highest percentages of AEs that were felt to be associated with CRS3123 treatment were for headache (6 [20.0%]), decreased

**TABLE 2** Summary of adverse events occurring in two or more subjects in a cohort<sup>a</sup>

Characteristic	Cohort A (100 mg, n = 6)	Cohort B (200 mg, n = 6)	Cohort C (400 mg, n = 6)	Cohort D (800 mg, n = 6)	Cohort E (1,200 mg, n = 6)	Placebo cohort (n = 10)
Abnormal urinalysis	3 (50.0)	3 (50.0)	0	0	0	3 (30.0)
Positive urine leukocyte esterase	1 (16.7)	0	0	3 (50.0)	0	0
Urine WBCs	1 (16.7)	2 (33.3)	0	2 (33.3)	0	0
Urine RBCs	2 (33.3)	0	0	0	0	0
Urine nitrite (+)	2 (33.3)	1 (16.7)	0	1 (16.7)	0	0
Headache	2 (33.3)	1 (16.7)	0	1 (16.7)	2 (33.3)	2 (20)
Dizziness	0	0	0	0	2 (33.3)	0
Musculoskeletal pain	0	1 (16.7)	0	2 (33.3)	2 (33.3)	2 (33.3)
Abdominal pain	0	0	0	2 (33.3)	1 (16.7)	0
Decreased hemoglobin	3 (50.0)	2 (33.3)	1 (16.7)	1 (16.7)	0	0
Increased neutrophils	0	0	0	0	2 (33.3)	0
Increased eosinophils	0	2 (33.3)	1 (16.7)	0	0	0
Decreased serum calcium	1 (16.7)	2 (33.3)	1 (16.7)	0	0	0

<sup>a</sup>Values are numbers (%).



**FIG 1** Composite of estimated concentrations of CRS3123 following five escalating doses. The concentrations were determined using the parent drug as the standard, but the concentrations depicted are for three molecular species combined, the parent drug and two of the three glucuronide metabolites that coelute with the parent drug. The parent drug constitutes an unknown percentage of the depicted concentrations. Black, cohort A, 100 mg; red, cohort B, 200 mg; blue, cohort C, 400 mg; green, cohort D, 800 mg; orange, cohort E, 1,200 mg. Note that absorption was not dose proportional and that relative bioavailability after the 1,200-mg dose was less than that after the 800-mg dose.

hemoglobin (5 [16.7%]), abnormal urine analysis (5 [16.7%]), and decreased blood calcium (4 [13.3%]). For AEs associated with placebo treatment, the highest percentage was for abnormal urine analysis (2 [20%]). Of the 30 subjects who received active drug across all cohorts, 7 (23.3%) experienced decreased hemoglobin compared to none in the placebo group. This adverse event was seen primarily in the lower-dose cohorts, in which 5 of 12 (43.3%) had decreased hemoglobin. However, all these events were classified as grade 1, with a decrease of less than 1 g/dl from baseline. Four subjects who received study product experienced decreased calcium levels, all on the day after product administration. All were grade 1, between 8.2 and 8.4 mg/dl, with the lower limit of normal being 8.5 mg/dl.

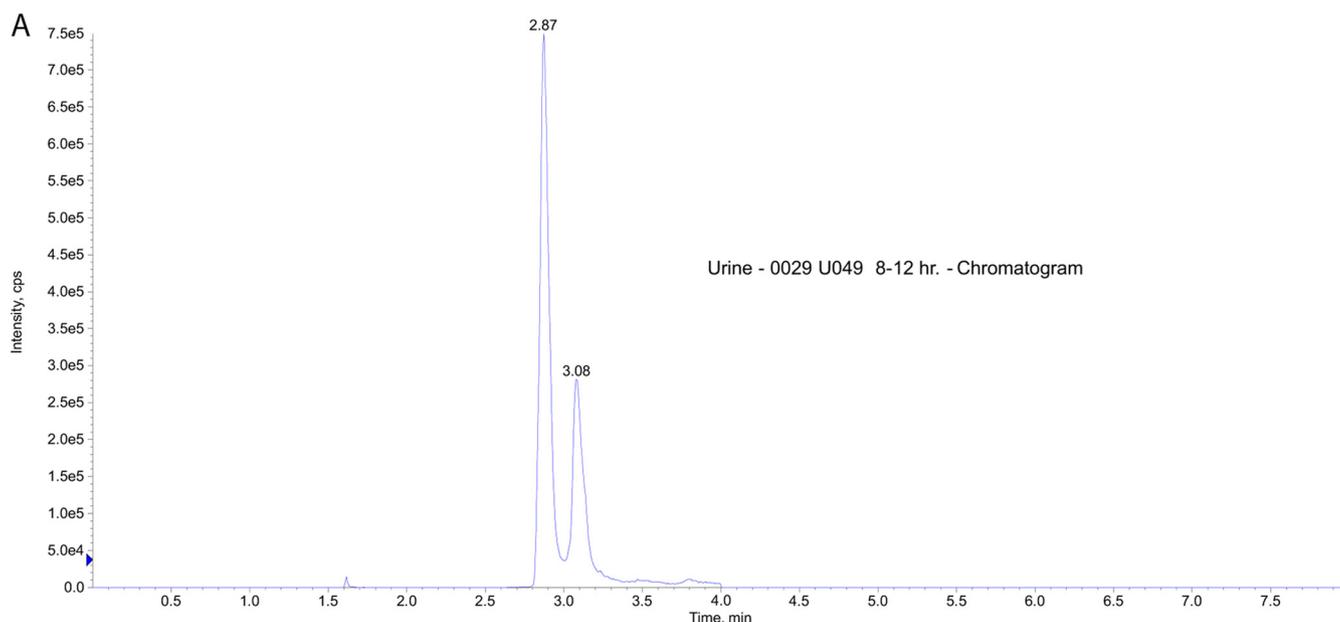
**Absorption of CRS3123.** After oral administration, some CRS3123 systemic exposure was observed. Quantitation was confounded when careful examination of the chromatographic peak revealed the presence of coeluting compounds. These peaks were present only in clinical samples and were presumed to be metabolites (see below). The lack of availability of analytical standards for these unanticipated metabolites precluded the separation and precise quantitation of all molecular species.

For the purpose of comparison, the areas of the peaks eluting at the time of the parent drug were assumed to be CRS3123 and assigned a relative concentration on this basis (Fig. 1). Concentrations in plasma peaked at 2 to 3 h after dosing and declined rapidly over 12 h. Absorption did not appear to be dose proportional, and the relative bioavailability appeared to decline with increasing dose. Both parent drug and metabolites were excreted in the urine (Fig. 2).

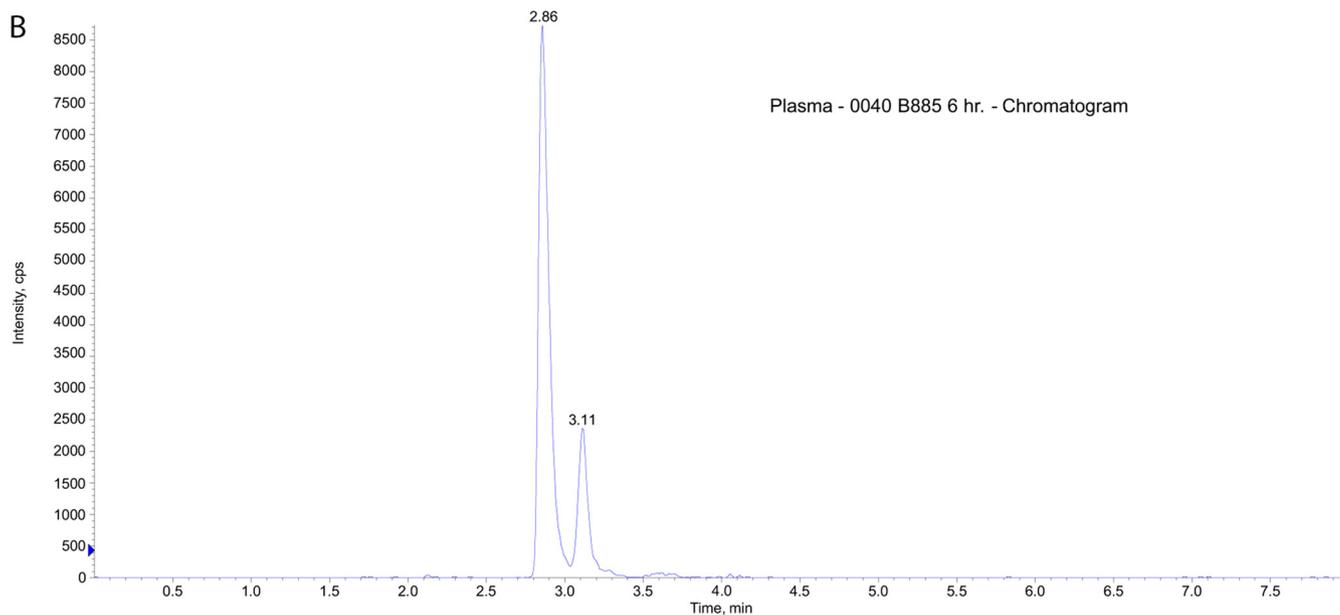
**CRS3123 metabolites.** All the plasma and urine samples contained a second peak that was resolved from the peak that eluted at the time expected for CRS3123 (Fig. 2). No standards or quality control samples (including samples of drug simply incubated under various conditions in standard plasma or urine) contained an extra peak.

The presence of two bromine atoms in the chemical composition of CRS3123 (Fig. 3) created a characteristic mass spectral pattern, as the two isotopes of bromine,  $^{79}\text{Br}$  and  $^{81}\text{Br}$ , occur in an approximately 1:1 ratio, and a compound containing two bromine

■ XIC of +MRM (2 pairs): 514.000/290.900 Da ID: C3123 from Sample 17 (Urine U049) of C3123 Urine25Aug2014.wiff (Turbo Spray) Max. 7.5e5 cps.



■ XIC of +MRM (2 pairs): 514.000/290.900 Da ID: c3123 from Sample 63 (0040 B885 6hr) of C312304-30-2014.wiff (Turbo Spray), Smoothed Max. 8732.0 cps.



**FIG 2** LC/MS chromatograms of urine (A) and plasma (B) that show that CRS3123 was metabolized after absorption and excreted as two molecular species. Such modification was not present in the CRS3123 standard chromatogram (C). The parent drug eluted at 2.87 min and a metabolite at 3.08 min. Mass spectral analysis showed that the mass of the slower-eluting molecule (3.08 min.) corresponded to an addition of 176 mass units, the mass of a hexuronide substitution, to CRS3123. As glucuronic acid is the only plausible hexuronide, an increase of 176 mass units could be attributed to the addition of a glucuronide to the CRS3123 molecule. Cleavage fragments at masses 554, 556, and 558, attributable to 0,2A cross-ring cleavage, confirmed the glucuronate moiety. All clinical samples contained the second peak (A and B), which was not present in the standard (C).

atoms will generate three mass peaks differentiated by 2 atomic mass units (amu) due to the three possible combinations of bromine isotopes that can occur in any one molecule ( $^{79}\text{Br } ^{79}\text{Br}$ ,  $^{79}\text{Br } ^{81}\text{Br}$ , or  $^{81}\text{Br } ^{81}\text{Br}$ ).

The mass spectrum from the unidentified peak was that of a compound containing two bromine atoms (3 peaks each differentiated by 2 mass units) at masses 688, 690, and 692. This corresponds to an addition of 176 mass units, the mass of a hexuronide substitution, to CRS3123. An increase of 176 mass units could be attributed to the addition of a glucuronide to the CRS3123 molecule.

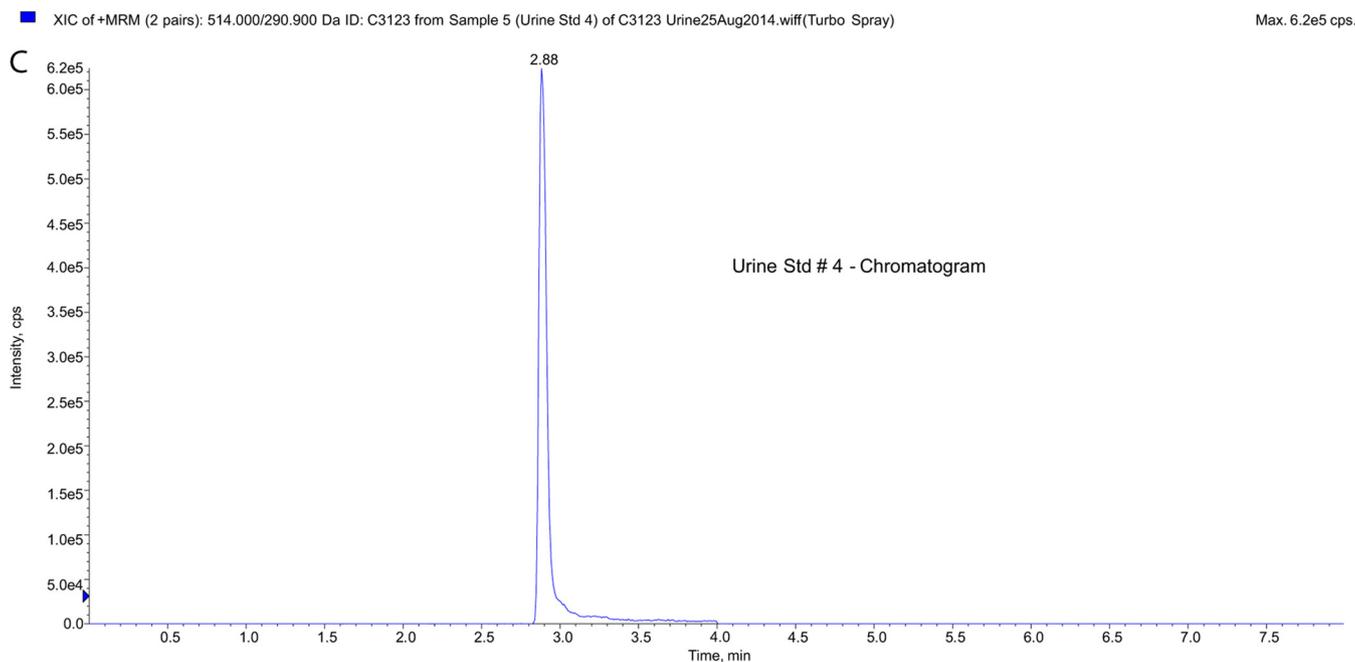


FIG 2 (Continued)

Further analysis of the spectra revealed that the 688, 690, and 692 masses were also present in the CRS3123 chromatographic peak, consistent with the presence of at least two “CRS3123-glucuronides,” one of which coeluted with CRS3123 under these chromatographic conditions. Since the spectra of “CRS3123-glucuronides” also contain the 514 mass used in the quantitation of CRS3123 in Fig. 1, it is reasonable to assume that coelution affected the quantitation of CRS3123.

To confirm these findings, the liquid chromatography(LC) gradient was modified (Table 3) to separate the coeluting CRS3123 and the presumed “CRS3123-glucuronide” peaks (Table 3). A change also was made to the tandem mass spectrometry (MS/MS) parameters. The monitoring of the 690.3 > 514.0 product ion was added so that peaks

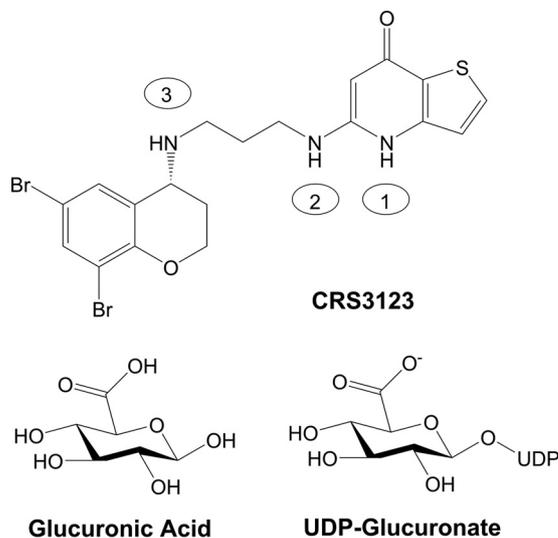


FIG 3 Structure of CRS3123. The three putative reaction sites for the enzymatic conversion of CRS3123 to *N*-glucuronide by conjugation with UDP-glucuronate are indicated by the numbers 1, 2, and 3 (circled). 1 and 2 are secondary aliphatic amines, and 3 is a heterocyclic amine. The formation of glucuronides could be expected at these sites.

**TABLE 3** Liquid chromatography gradient programs<sup>a</sup>

Initial program			Modified program		
Time (min)	% A	% B	Time (min)	% A	% B
0.00	0.00	100	0.00	30	70
0.57	0	100	1.57	30	70
1.57	95	5	3.00	40	60
4.00	95	5	4.00	95	5
5.00	0	100	8.00	95	5
8.00	0	100	9.00	30	70
			11.00	30	70

<sup>a</sup>Reservoir A contained LC/MS methanol; reservoir B contained 0.05% acetic acid in HPLC H<sub>2</sub>O; the HPLC column was Fortis Diphenyl, 50 by 2.1 mm (inner diameter) by 5 μm; the flow rate was 0.425 ml/min.

attributable to “CRS3123-glucuronide” could be differentiated from the CRS3123 peak. Since “CRS3123-glucuronide” has both mass 690.3 and mass 514.0 in its spectrum, it will yield peaks in both the 690.3 > 514.0 multiple-reaction monitoring (MRM) transition and the 514.0 > 290.9 MRM transition. However, CRS3123 has no mass 690.3 and will produce a peak only in the 514.0 > 290.9 MRM transition.

A third MRM transition, 690.3 > 556.0, also was added, as the two spectra of the “CRS3123-glucuronides” differed in the presence of masses at 554, 556, and 558. The 690.3 > 556.0 MRM transition would ensure that the differences in their mass spectra were due to structural differences between the two “CRS3123-glucuronides” that resulted in unique fragmentation patterns and not due to the presence of another metabolite. If the masses at 554, 556, and 558 are fragmentation ions of the “CRS3123-glucuronide,” the 690.3 > 556.0 MRM transition should have a chromatographic peak at the same retention time as the parent compound. If, however, the 554, 556, and 558 masses are due to the presence of another compound, then the 690.3 > 556.0 MRM transition will not generate a peak.

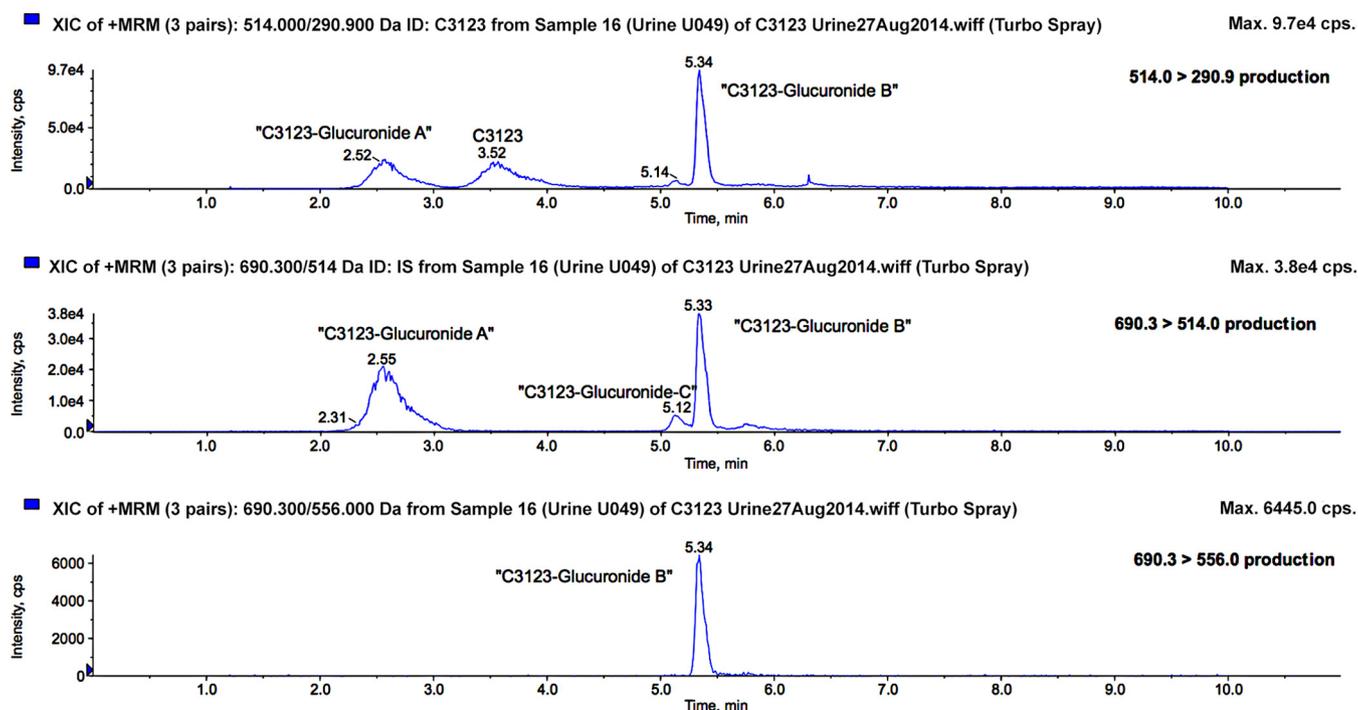
The resulting chromatogram shows the separation of CRS3123 and the “CRS3123-glucuronide” peaks (Fig. 4). The 690.3 > 556.0 MRM transition produced a peak with the same retention time as “CRS3123-glucuronide.” This provides strong evidence that the mass fragments at 554, 556, and 558 observed in the second “CRS3123-glucuronide” peak are part of that compound’s fragmentation pattern and not another metabolite. The mass fragments at 554, 556, and 558 may be attributable to a 0,2A cross-ring cleavage of the glucuronate moiety. The modified LC gradient also revealed the possible existence of a third metabolite labeled “CRS3123-glucuronide-C” (Fig. 4).

To determine whether a different column would produce similar chromatography, we replaced the Fortis Diphenyl column (50 by 2.1 mm) used during the analysis of study samples with a Discovery C<sub>18</sub> column (50 by 2.1 mm). The LC parameters were unchanged. The resulting chromatogram showed the same coelution of CRS3123 and a “CRS3123-glucuronide” along with the emergence of the second peak.

Glucuronidation began with the appearance of CRS3123 in plasma, as seen for glucuronide B in Fig. 5B, and concentrations of this metabolite paralleled those of the parent drug and the two glucuronides that coeluted with it (Fig. 5A). Excretion of the glucuronides in urine appeared to lag somewhat behind their appearance in plasma. They were present in all urine samples, but because urine samples were not collected simultaneously with plasma samples, direct comparisons between the two could not be made.

## DISCUSSION

There is a critical need for new therapies for CDI, and CRS3123 appears to be a promising candidate. By targeting methionyl-tRNA synthetase, CRS3123 inhibits protein synthesis and prevents toxin production and sporulation in *C. difficile*. It not only should be an effective treatment for *C. difficile* but also should prevent recurrence of disease, persistent colonization, and horizontal transmission. Consistent with this notion, CRS3123 was more effective in the hamster model of *C. difficile* infection than either metronidazole or vancomycin (18).

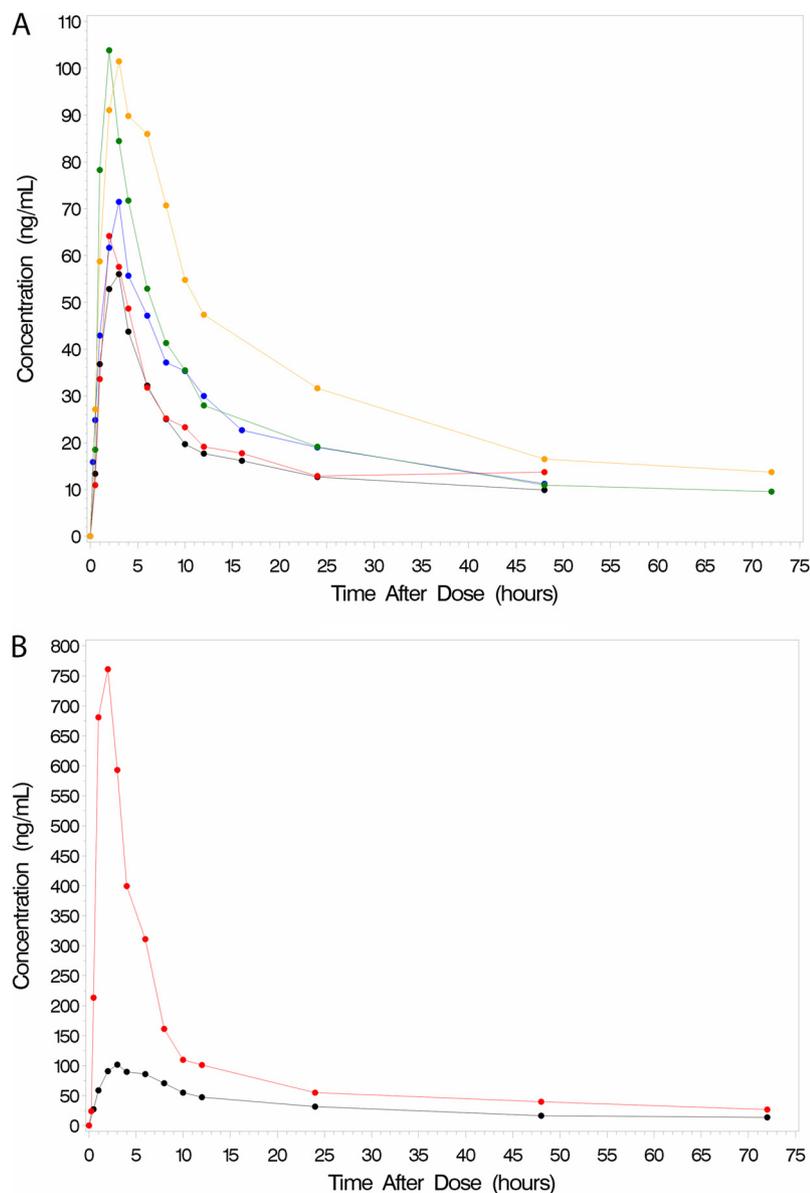


**FIG 4** LC/MS/MS spectra of a urine specimen with use of the modified LC program summarized in Table 3. The specimen was chosen for its available volume. The three panels are spectra from three different MRM transitions. (Top) MRM transition between  $m/z$  514 and 290.9 product ion. The mass of CRS3123, 514 Da, is within the range of this MRM transition, and it produces a peak at 3.52 min. Three additional peaks are found at 2.52 (glucuronide A), 5.14 (glucuronide C), and 5.34 (glucuronide B) minutes. Glucuronides A and C coelute with the parent compound, as illustrated in Fig. 1 and 2. (Middle) MRM transition between  $m/z$  690.3 and 514.0 product ion. Since CRS3123 has no mass of >514 Da, a peak representing the parent drug is absent, but peaks representing glucuronides A (2.55 min), C (5.12 min), and B (5.33 min), which have nominal masses of 690 Da, are present. (Bottom) MRM transition between  $m/z$  690.3 and 556.0. Glucuronide B is present at 5.34 min. The presence of the parent compound in the top panel shows that glucuronidation is not required for renal clearance.

Bioavailability of CRS3123 in rats and dogs was assessed without regard for metabolites that were not known at the time. In both species, bioavailability decreased as the dose increased, and it ranged from <1% to 10.5% (rat) and 7.3% (dog) (Crestone Inc., unpublished). Bioavailability following oral administration to humans could not be accurately assessed, because of the absence of authentic standards for the metabolites. A fraction of the administered dose of CRS3123 was absorbed, and as with rats and dogs, bioavailability after lower oral doses was greater than that after higher doses.

We have detected and characterized several of the most prevalent metabolites. Compounds with secondary aliphatic amines and heterocyclic amines are often metabolized into *N*-glucuronides in humans. Since CRS3123 contains two secondary aliphatic amines and a heterocyclic amine, the formation of glucuronides would be likely. Figure 3 shows the structures of CRS3123, glucuronic acid, and UDP-glucuronate. The circled numbers (1, 2, and 3) show possible reaction sites for the enzymatic conversion of CRS3123 to *N*-glucuronide by conjugation with UDP-glucuronate at three separate positions. The addition of 176 mass units to CRS3123 is consistent with the addition of a hexuronic acid, and the mass fragments at 554, 556, and 558, attributable to 0,2A cross-ring cleavage, are consistent with cleavage of a glucuronate moiety. Glucuronidation may increase renal excretion, although absorbed CRS3123 was excreted in urine both in its native state and after glucuronidation, so the modification does not appear to be needed for renal clearance. Glucuronides are rarely biologically active, so their presence is not likely to result in increased toxicity or biological activity. Further investigation will be required, although the majority of the dose likely remains localized to the gastrointestinal tract.

This study was the first-in-human assessment of CRS3123. We found that single escalating doses of CRS3123 administered orally to healthy research participants were safe and well tolerated over the investigated dose range. AEs were similar in severity



**FIG 5** Composite of glucuronide B concentrations in plasma (A) and comparison with the concentration of the three molecular species, combined, that coelute as the “parent drug” (B). (A) Comparative mean glucuronide B concentrations by protocol time for cohorts A to E. The concentrations were determined using the parent drug as standard; since the mass of glucuronide B is 176 mass units greater than that of the parent drug, use of the parent drug as the standard overestimates the concentrations of the glucuronide. Black, cohort A, 100 mg; red, cohort B, 200 mg; blue, cohort C, 400 mg; green, cohort D, 800 mg; orange, cohort E, 1,200 mg. Note that the concentrations of glucuronide B paralleled those of the parent drug in Fig. 1. (B) Mean concentrations of parent drug and glucuronide B, over time, in cohort E. Note that glucuronidation began with absorption and the peak concentration of glucuronide B coincided with that of the parent drug.

and frequency for participants who received active drug and for those who received placebo. All AEs were mild to moderate except for a single severe event of proteinuria in the placebo group.

Although safe and well tolerated at all doses tested in this study, doses at the higher end of the range are expected to be supratherapeutic and not be needed for the treatment of *C. difficile*, since most of the drug is not absorbed. A more detailed understanding of both systemic exposure and fecal concentrations of the drug will be gained in a separate planned multiple-ascending-dose phase 1 clinical trial. In the

phase 2 trial, systemic exposure will need to be reassessed since compromised intestinal epithelium in CDI patients could affect the degree of absorption of the drug.

It is worth noting that the CRS3123 concentrations in plasma after the higher doses were well in excess of the MICs of most clinically important Gram-positive cocci, and consideration should be given to development of the compound for the treatment of infections caused by these bacteria, with appropriate account of exposure-response metrics.

This first assessment of CRS3123 in humans provides impetus to further evaluate this promising agent as a novel treatment option for CDI.

## MATERIALS AND METHODS

We conducted a double-blind, single-center, placebo-controlled dose escalation study of CRS3123 in healthy adults. Our dose range was 100 mg (cohort A), 200 mg (cohort B), 400 mg (cohort C), 800 mg (cohort D), and 1,200 mg (cohort E). The starting dose was based on a 100-fold margin over the no-observable-adverse-effect levels (NOAELs) in rat and dog toxicology studies and on the projected effective human dose.

**Study design.** We conducted the study between 6 June 2012 and 6 May 2014 in the inpatient research unit at Johns Hopkins Bayview Medical Center (JHBMC). Research participants were randomized in five sequential dose-escalating cohorts of eight participants. In cohorts A to D, eight research participants were randomized to receive either active drug or placebo in a 3:1 ratio. In cohort E, the first two research participants were randomized in a 1:1 ratio to provide a sentinel cohort, and the remaining six participants were randomized in a 5:1 ratio, yielding an overall 3:1 ratio. The study was approved by the Johns Hopkins University Institutional Review Board, and written informed consent was obtained from all research participants prior to study screening. Eligible research participants were healthy adults who met the criteria of the NIH Healthy Volunteer policy (20). Briefly, healthy research participants were 18 to 45 years old, with a body mass index less than 35 and a negative illicit-drug screen, and if sexually active, using adequate contraception. Exclusion criteria included history of a chronic medical condition, severe allergic reaction to any medication, pregnancy, and active drug or alcohol dependence. Research participants who had any history of CDI, received any blood products in the 6 weeks before study day 0, or were taking any prescription medication other than oral contraceptives in the previous 30 days were also excluded. Additional exclusion criteria were abnormal findings on standard chemistry, hematology, and urinalysis profiles, positive serology for hepatitis B, hepatitis C, or HIV, or prolongation of the corrected QT (QTc) interval on electrocardiography. Subjects participated in the study for approximately 9 weeks, including a 4-week screening period, a 3-day inpatient stay, and approximately 4 weeks of follow-up after administration of CRS3123.

The investigational drug was formulated in hard gelatin capsules containing CRS3123 hydrochloride in either 100-mg or 200-mg doses and inert compendial ingredients (microcrystalline cellulose, sodium starch glycolate, and magnesium stearate). Matching placebo capsules contained only the inert excipients. Study drug and placebo were maintained in the Investigational Drug Pharmacy at JHBMC and dispensed by the unblinded study pharmacist.

On the day before dosing, research participants were admitted to the clinical research unit. A complete medical history and physical examination were obtained along with samples for baseline lab tests, including complete blood count, serum chemistries, urinalysis, urine toxicology screen, and pregnancy test.

Prior to dosing on day 0, vital signs were recorded and ECGs were obtained in triplicate to measure baseline QTcB intervals. After review of the participant's diary card for any symptom from the previous 24 h, the pharmacist dispensed the appropriate capsules based on the specific cohort. The study drug was orally administered to 12-h-fasted participants with a minimum of 8 oz water, and participants continued fasting until 2 h after drug administration. Participants were allowed to drink water before and after administration.

Drug administrations were observed by a study physician, and vital signs were closely monitored. ECGs were obtained at 1, 4, 8, and 24 h postdose. Blood and urine samples were collected at specified times. Participants were discharged the following day and monitored with outpatient visits on days 2, 3, 7, 14, and 28.

An independent safety monitoring committee reviewed data through day 7 for cohorts A through C before escalation to cohort D and reviewed the cohort D data before escalating to cohort E.

**Safety analyses.** Adverse events reported by participants on diary cards or observed by a study physician were assessed by interview, physical examination, and review of clinical safety labs drawn from the time of study drug administration through day 28. AEs were graded for severity as mild (grade 1, requiring minimal or no treatment and not interfering with the patient's daily activities), moderate (grade 2, resulting in a low level of inconvenience or concern and with therapeutic measures), severe (grade 3, interrupting a participant's usual daily activity and possibly requiring systemic therapy or other treatment), or life threatening (grade 4, placing the participant at immediate risk of death from the reaction as it occurred). The relationship of an AE to the study drug was assessed as related or not related based on the temporal relationship of the event to the administration of the investigational product, identification of an alternative etiology, and biological plausibility.

Analyses of safety labs were conducted at the JHBMC clinical laboratory. Safety laboratory tests included complete blood counts, including leukocyte differential, comprehensive metabolic panels

(sodium, potassium, chloride, calcium, carbon dioxide, creatinine, glucose, blood urea nitrogen, creatine phosphokinase [CPK], aspartate aminotransferase [AST], alkaline phosphatase [AP], alanine aminotransferase [ALT], total bilirubin, protein, albumin, amylase), and urinalyses (protein, blood, pH, and specific gravity). Laboratory tests found to be outside normal ranges and reaching grade 1 were reported as AEs.

**Measurement of CRS3123 in plasma and urine.** Blood samples for CRS3123 quantitation were obtained before dosing and at the following times after dosing: 15 and 30 min, and 1, 2, 3, 4, 6, 8, 10, 12, 16 (all cohorts), 24, 48, and 72 h (cohorts D and E). A 24-h timed urine collection was obtained after dosing at the following intervals: 0 to 2 h, 2 to 4 h, 4 to 8 h, 8 to 12 h, and 12 to 24 h.

Six-milliliter blood samples were drawn into collection tubes containing sodium heparin and placed immediately on ice. The blood was centrifuged at  $2,500 \times g$  within 2 h of collection, and the plasma fraction was removed, divided into two aliquots, and frozen at  $-70^{\circ}\text{C}$  for shipment to the Analytical Pharmacology Laboratory at the University of Toledo. For analysis, plasma samples were allowed to thaw on ice in a covered ice bucket for protection from light, and once thawed, a  $50\text{-}\mu\text{l}$  aliquot was transferred to a labeled tube on ice. The remainder of the sample was refrozen immediately at  $-70^{\circ}\text{C}$ .

All bioanalytical standard and stock solutions were stored at  $-70^{\circ}\text{C}$  and equilibrated to ambient temperature before use. To correct for purity, the weight of the compound obtained from the analytical balance was multiplied by the purity to yield the actual weight.

To a 1.5-ml labeled microcentrifuge tube containing  $50\ \mu\text{l}$  of the internal standard (REP8839; Crestone, Inc. [21] working solution, 250 ng/ml ammonium formate in LC/MS methanol and  $50\ \mu\text{l}$  of subject plasma were added and mixed for 30 s. Following mixing, the sample was diluted with  $100\ \mu\text{l}$  1% ammonium formate in LC/MS methanol, mixed on a vortex mixer for 30 s, and centrifuged at 16,400 rpm for 15 min at room temperature. Then,  $150\ \mu\text{l}$  of the supernatant was transferred by pipette to an autosampler vial for injection.

Detection and analysis were performed with a validated LC-MS/MS assay adapted using an Agilent 1100 binary pump, column heater, and autosampler (Santa Clara, CA) interfaced with an API 3000 Sciex Mass Spectrometer Model 018444-S (Framingham, MA). The autosampler injection volume was  $10\ \mu\text{l}$  onto a 50 by 2.1 mm (inner diameter) by  $5\ \mu\text{m}$  Fortis Diphenyl high-performance liquid chromatography (HPLC) column heated to  $32^{\circ}\text{C}$ . The gradient for elution was comprised of LC/MS methanol and 0.05% acetic acid in HPLC water in the two reservoirs, respectively. The solvent flow rate was 0.425 ml/min with a total run time of 8.0 min. The gradient was programmed to start at 0% methanol for 57 s, and then the methanol concentration increased to 95% over the next minute. The methanol concentration was maintained until 4 min after injection, after which it was decreased to 0% over the next minute and maintained until the end of the run. To minimize the amount of carryover, a rinse was performed between samples. The rinse employed the same mass spectrometer parameters but a slightly different gradient and a blank methanol injection. After injection of the blank methanol, the rinse began with 0% methanol at 0.450 ml/min for 17 s. The concentration of methanol then increased linearly to 95%, reaching its plateau at 57 s. This was maintained until 2 min 50 s after the injection, whereupon the concentration of methanol was decreased linearly to 0% by 4 min. The solvent flow rate was then decreased to 0.425 ml/min for the remaining 2 min of the wash.

Selective ion monitoring for CRS3123 followed the transition of the parent ion at 514.10 atomic mass units (amu) to the transition ion at 290.90 amu and for the internal standard, REP8839, the transition of the parent ion at 450.10 amu to the transition ion at 232.90 amu. Quantitation was performed based on peak area ratios with upper and lower limits of quantitation of 3,000 ng/ml and 25 ng/ml, respectively.

The method for the detection and quantitation of CRS3123 in urine was identical to that for plasma. The upper and lower limits of quantitation were 8,000 ng/ml and 100 ng/ml, respectively.

No metabolites of CRS3123 were known prior to human dosing, and the bioanalytical methods were validated only for the parent drug in plasma and urine. All clinical samples contained an extra peak with baseline resolution from the peak chromatographically eluting at the time expected for CRS3123.

To initially characterize the second peak, mass spectra were collected from study samples. Two samples, one from the 4- to 8-h collection window and one from the 8- to 12-h collection window, were chosen because of the large second peak observed during sample analysis. Urine samples were selected in preference to plasma samples because of the larger remaining volume of sample. Mass spectral analysis of urine samples identified two metabolites that coeluted with the parent drug.

**Statistical analyses.** Categorical variables were summarized as numbers and percentages of subjects and continuous data with standard deviations, means, and medians.

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