Cadazolid Does Not Promote Intestinal Colonization of Vancomycin-Resistant Enterococci in Mice

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The promotion of colonization with vancomycin-resistant enterococci (VRE) is one potential side effect during treatment of *Clostridium difficile*-associated diarrhea (CDAD), resulting from disturbances in gut microbiota. Cadazolid (CDZ) is an investigational antibiotic with potent *in vitro* activity against *C. difficile* and against VRE and is currently in clinical development for the treatment of CDAD. We report that CDZ treatment did not lead to intestinal VRE overgrowth in mice.

Current antibiotic treatment of *Clostridium difficile*-associated diarrhea (CDAD) is mostly done with oral metronidazole (MDZ) and vancomycin (VAN), while only VAN is approved by the FDA. Both drugs promote overgrowth of vancomycin-resistant enterococci (VRE) in the gut and long-term colonization on treatment (1). Recently, the antibiotic fidaxomicin (FDX) was approved by the FDA as a new treatment option for CDAD. Its clinical impact on VRE overgrowth is still unclear. Colonization with VRE represents a major source for VRE bloodstream infections, endocarditis, and urinary tract infections, a particular problem in intensive care units (ICUs) (2–5). Infections caused by VRE are more serious and are associated with a higher mortality rate than those caused by vancomycin-sensitive enterococci (6–9). VRE control appears to be highly challenging. Therefore, preventing VRE colonization represents an important health care goal, particularly in the ICU.

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FIG 1 Mice were pretreated with cadazolid, vancomycin, fidaxomicin, or metronidazole once daily at the indicated doses per kilogram body weight per day (vehicle-treated group, 0 mg/kg) from day –2 to day +2 and infected with VRE on day 0. Results are expressed as change in log CFU per cecum (versus vehicle group) at 3 days postinfection. Shown are 25% to 75% interquartile range (box), median (intersection), and minimum–maximum (t bar) values for each group. Data were pooled from two similar, independent experiments. API, active pharmacological ingredient. *, $P < 0.05$; ***, $P < 0.001$.

FIG 2 Mice were precolonized with VRE for 7 days and treated with cadazolid, vancomycin, fidaxomicin, or metronidazole for 4 days once daily at the indicated doses per kilogram body weight per day (vehicle-treated group, 0 mg/kg). Results are expressed as change in log CFU per cecum (versus vehicle group) at 24 h after 4 days of treatment. Shown are 25% to 75% interquartile range (box), median (horizontal line), and minimum-maximum (t bar) values for each group. Data were pooled from three similar, independent experiments. API, active pharmacological ingredient. ***, $P < 0.001$.

Cadazolid (CDZ; ACT-179811), a new investigational antibiotic in development for the treatment of CDAD (10), exhibited efficacy and potency in prevention of CDAD similar or superior to VAN, MDZ, and FDX in the mouse model for CDAD (10, 11). CDZ exhibited potent *in vitro* activity not only against *C. difficile* clinical isolates but also against VRE (MIC₉₀, 2 µg/ml) (12, 13), while having a limited impact on bacteria of the normal gut microflora in the *in vitro* human gut model (14). Recently, a phase 2 trial in CDAD showed clinical cure rates with CDZ treatment similar to those with VAN treatment, while having lower recurrence rates, resulting in higher sustained cure rates (15).

In order to investigate the impact of CDZ on intestinal VRE expansion, a mouse model for VRE colonization was employed (1; see Supplementary Material and Methods in the supplemental material). All animal housing and experiments were conducted in agreement with the Swiss Federal Ordinance for animal protection, the animal welfare guidelines from the Cantonal Veterinary Office Basellandschaft, and the Actelion Pharmaceuticals, Ltd., internal animal welfare guidelines. Identical doses that led to a 100% effective dose (ED₁₀₀) in the prevention of murine CDAD were used to investigate the impact of CDZ on VRE colonization in mice. Doses for comparator treatments were also chosen according to their ED₁₀₀ in the prevention of murine CDAD (where ED₁₀₀ represents the dose for the effect of prevention of death in 100% of the mice during experimental *C. difficile* infection (see supplemental material; 10). Mice were treated with investigated antibiotics and then challenged orally with VRE strain A-949 (VanA⁺/VanB⁻, clinical isolate obtained from the University

Hospital of Basel, Switzerland (see Supplementary Material and Methods in the supplemental material), and VRE counts were determined by plating cecal samples on Enterococcosel agar plates (BD) containing VAN (8 µg/ml) 3 days postinfection.

As shown in Fig. 1, CDZ pretreatment caused no increase in VRE counts ($P > 0.05$ versus vehicle group; CFU counts were subjected to Kruskal-Wallis analysis of variance [ANOVA], followed by Mann-Whitney U posthoc significance test), and an intestinal VRE expansion was prevented. Colonies growing *ex vivo* on plates had the same colony morphology and MIC profile as the original VRE strain used for infection. Furthermore, no VRE colonies were detected in noninfected mice (data not shown). It is highly probable that VRE colonies detected after treatment were from the same strain that was used to inoculate the mice. Similar results were obtained with FDX ($P < 0.05$ for the 10-mg/kg group; $P > 0.05$ for all other groups). In contrast, pretreatment with VAN ($P < 0.001$ for all groups) or MDZ ($P < 0.001$ for all groups) greatly increased VRE counts.

The ability of CDZ to prevent intestinal VRE expansion in mice may be due to the potent *in vitro* activity of CDZ against VRE, potentially preventing or suppressing intestinal VRE growth. To investigate this, mice were established with a VRE colonization for 1 week following the procedure of Ubeda et al. (16) based on a perturbation of the endogenous gut flora by an ampicillin treatment (see Supplementary Material and Methods in the supplemental material). After 1 week of establishment of VRE colonization, mice were treated with CDZ at the same doses, i.e., the doses conferring ED₁₀₀ in prevention of murine CDAD for 4 days. As

TABLE 1 MIC of VRE clones isolated before and after *in vivo* treatment

Compound ^a	MIC before treatment (μg/ml)	MIC (μg/ml) ^b after 4 days of treatment with compound ^c at the following dose (mg/kg):												
		Vehicle	CDZ				VAN		MDZ		FDX			
			1	10	30	100	30	100	300	900	1	10	30	100
CDZ	0.5	0.5	0.5	0.25–0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
VAN	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	
MDZ	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	
FDX	1–2	1–2	1–2	1–2	1–2	1–2	2	2	2	2	1–2	1–2	2–8	64

^a Compound used for MIC testing.^b MICs (range) of colonies obtained *ex vivo* from experiments shown in Fig. 2.^c Compound used for *in vivo* treatment.

shown in Fig. 2, VRE counts were dose dependently reduced by CDZ treatment (P value of <0.001 for all doses, compared with the vehicle group). Such reduction was not observed after treatment with VAN or MDZ ($P > 0.05$ for all groups), in agreement with a lack of *in vitro* activity against VRE. No VRE reduction was observed for FDX treatment despite intrinsic activity against the VRE strain used ($P > 0.05$ for all groups).

In order to monitor susceptibility of VRE during treatment, MICs of VRE colonies recovered *ex vivo* were determined before and after treatment. As shown in Table 1, VRE MICs for CDZ were low (0.5 μg/ml) and did not change after *in vivo* treatment with any compound. In contrast, MICs for FDX increased from 1 μg/ml to 64 μg/ml during FDX treatment, indicating the development of resistance to FDX or expansion of a preexisting FDX-resistant subpopulation, thus explaining the lack of efficacy in reducing VRE counts *in vivo*. As expected, the VRE strain was not susceptible to VAN or MDZ.

The data presented here indicate that CDZ does not exhibit a risk for colonization with VRE. CDZ even exhibited high efficacy in reducing an established preexisting VRE colonization. This finding correlates well with the potent *in vitro* activity of CDZ against VRE and the low propensity of resistance development (12). The present results are also in line with a low impact of CDZ on the endogenous gut flora (14), possibly minimizing the creation of a niche used by VRE for colonization. The extent of the contribution of the gut flora sparing of CDZ to the low risk for VRE colonization in mice remains to be tested. The data also confirm *in vivo* the low propensity of resistance development of CDZ observed *in vitro* (12).

The presented results on VAN and FDX are in line with published results on VRE colonization in mice (17–19). Both of the current first-line treatment options for human CDAD, VAN and MDZ, are associated with risk factors for VRE colonization also in patients (1, 20, 21). Emergence of FDX-resistant VRE during therapy has been observed in animal models and in clinical use (20, 22). The relevance of the low-risk association of CDZ with VRE colonization in mice for a low risk for VRE acquisition and overgrowth during treatment of CDAD in humans remains to be confirmed in future clinical trials.

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