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A Novel Agent Effective against *Clostridium difficile* Infection

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N^2-(3,4-Dichlorobenzyl)-7-(2-[1-morpholinyl]ethyl)guanine (MorE-DCBG, 362E) is a synthetic purine that selectively inhibits the replication-specific DNA polymerase of *Clostridium difficile*. MorE-DCBG and its analogs strongly inhibited the growth of a wide variety of *C. difficile* strains. When administered orally in a hamster model of *C. difficile*-specific colitis, 362E was as effective as oral vancomycin, the current agent of choice for treating severe forms of the human disease.

Clostridium difficile is an anaerobic, spore-forming, Gram-positive bacterium. It is the causative agent of *C. difficile*-associated disease (CDAD), an increasingly common, life-threatening disease (5, 6, 8). New anti-CDAD agents are needed to supplement those in current use. We have developed a series of 7-substituted-N^2-(3,4-dichlorobenzyl)guanines (DCBGs) that selectively inhibit the DNA polymerase IIIC (pol IIIC) which *C. difficile* requires to replicate its DNA (9). One of these derivatives, MorE-DCBG (362E) (structure in Fig. 1) displays excellent potential for further development as a clinical agent. Its properties are described below.

Inhibitors and antibacterial agents. Compound 362E and other DCBGs (structures in Fig. 1) were synthesized and purified as described previously (10, 11). Vancomycin and metronidazole were from Sigma (St. Louis, MO) and clindamycin from Spectrum (New Brunswick, NJ). For use in antimicrobial assays, compounds were prepared as 40 mM stock solutions in reagent-grade dimethyl sulfoxide (DMSO). Bacterial growth was not affected by DMSO concentrations up to 5%.

Assays of antibacterial activity. Assays were performed by Micromyx LLC (Kalamazoo, MI) and R. M. Alden Research Laboratory (Culver City, CA), using media and methods recommended by the Clinical and Laboratory Standards Institute for susceptibility testing of anaerobes (4).

Four derivatives of a larger number of N^2-substituted purines (9; data not shown) which displayed potent inhibitory activity against *C. difficile* pol IIIC (9) were assessed for their MICs versus 23 different *C. difficile* strains. These included one ATCC strain and 22 clinical isolates, several of which displayed different sensitivities to vancomycin and metronidazole. The results, summarized in Table 1, show that the ethyl analog, 362E, was clearly the most potent of the new compounds, displaying MIC_{50} and MIC_{90} values (2 and 4 μg/ml) close to those found for the comparators, vancomycin and metronidazole. The weaker 7-morpholinylalkyl-DCBGs were approximately equipotent. None of the vancomycin- and metronidazole-resistant strains used in this MIC assay displayed cross-resistance to any of the four DCBGs (data not shown).

Orally, 362E and 359E are poorly absorbed and apparently nontoxic. Given the results of the MIC experiments, we chose 362E and 359E for assessment of efficacy in the hamster CDAD model. Before proceeding, we examined their toxicity in hamsters and their absorption from the hamster gastrointestinal (GI) tract. (i) The results (not shown) indicate that oral doses of either compound as high as 1,000 mg/kg of body weight caused no obvious toxicity. (ii) GI absorption is an important determinant of an agent's potential for achieving a high local concentration at the site of *C. difficile* infection in the colon. The approach, liquid chromatography-mass spectrometry (LC-MS) quantification and classical area-under-the-curve pharmacokinetics, indicated that less than 5% of an oral dose of 75 mg/kg was absorbed in both cases (results not shown).

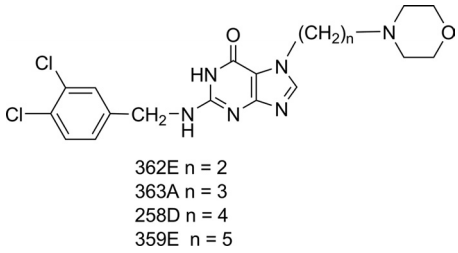

FIG 1 Structures of 7-(morpholinyl)alkyl-DCBGs.

Assay of drug efficacy *in vivo*. The golden Syrian hamster-based model of *C. difficile*-induced colitis described by Kokkotou et al. (7) was used. In this model, 80- to 90-g female golden Syrian hamsters are injected subcutaneously with a single dose (15 mg/kg) of clindamycin hydrochloride and, 24 h later, 1 ml of *C. difficile* spore suspension (ATCC strain 43255, 0.5 to 1 $\times$ 10^7 CFU/ml, prepared by us or by R. M. Alden Research Laboratory) is administered by oral gavage to each animal. In the absence of treatment, the infected animal soon develops diarrhea and dies within 48 to 66 h of infection. The development of the *C. difficile*-induced disease state depends on pretreatment with clindamycin; with no pretreatment, the animals remain healthy and do not develop CDAD.

To examine the efficacy of an agent in the model, suspensions of the agent in 1% carboxymethylcellulose (CMC) were administered by oral gavage to groups of 6 animals, starting 17 h after spore administration and continuing twice daily for 3 days (or more than 3 days, if indicated). The experiment also included a negative-control group receiving only vehicle and a positive-control group receiving oral vancomycin in the same regimen (7).

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TABLE 1 Activity of 7-morpholinylalkyl-DCBGs and comparators against 23 *C. difficile* strains[a]

Compound	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
359E	8	8
258D	4	16
363A	8	8
362E	2	4
Vancomycin	1	4
Metronidazole	1	4

[a] Experiments were conducted by R. M. Alden Research Laboratory with 22 clinical isolates and an ATCC strain.

To assess recurrent infection following various treatment periods, animals were maintained in their cages for up to 34 days after initial infection. Cages and their contents were changed every 7 days. The colonic contents of all animals that died were tested for the presence of *C. difficile* toxin A and/or B (Xpect *C. difficile* toxin A/B test kit; Remel, Inc., Lenexa, KS) according to the manufacturer's instructions.

Given the strong anti-*C. difficile* properties of 362E and 359E, their favorable toxicity profiles, and their marginal GI absorption, we examined their efficacy in the hamster CDAD model. The results, summarized in Table 2, indicate that oral treatment with vancomycin, 362E, or 359E at a dose of 50 mg/kg twice daily for 3 days completely protected infected animals for a period of up to 5 days. Under these conditions, 362E appeared to be more potent than 359E—for example, 6.25 mg/kg of 362E was superior to the same dose of 359E ($P < 0.001$; one-way analysis of variance [ANOVA]), whereas 6.25 mg/kg of 362E was not significantly different from 12.5 mg/kg of 359E ($P > 0.05$; Bonferroni multiple comparisons).

Prolongation of the treatment period reduces recurrence of CDAD. Although drug treatment at 50 mg/kg twice daily completely protected animals (Table 2), this protection did not persist when the observation period went beyond 5 days. Recurrent, lethal disease was observed in both the 362E and vancomycin groups—67% of treated animals died when treatment was limited to 3 days postinfection (Fig. 2). However, treatment for 7 days with 362E at 50 mg/kg twice daily reduced the recurrence rate to 40% and delayed death when the disease recurred, and when the same treatment regimen was continued for a total of 14 days, there was no recurrence observed during the remainder of the 34-day observation period (Fig. 2) (note that intestinal exudates of all animals that died were positive for toxin A and/or B and those of all survivors were negative). These results show that the efficacy of 362E in CDAD, like that of vancomycin, depends on the length of the period during which the drug is administered.

TABLE 2 Activity of test compounds on *C. difficile* infection model in golden Syrian hamsters

Group ($n = 6$)	Treatment,[a] mg/kg	No. of survivors at:			% Survivors at 120 h
		24 h	48 h	72 h	
1	None (negative control)	6	4	0	0
2	Vancomycin, 50	6	6	6	100
3	359E, 50	6	6	6	100
4	359E, 25	6	6	6	100
5	359E, 12.5	6	6	5	67
6[b]	359E, 6.25	1	1	1	0
7	362E, 50	6	6	6	100
8	362E, 25	6	6	6	100
9	362E, 12.5	6	6	6	100
10	362E, 6.25	6	6	6	83

[a] Treatment was *per os*, twice daily, for 3 days. Treatments were begun 16 to 18 h postinfection. All animals were pretreated with clindamycin hydrochloride (15 mg/kg, SC) 24 h before oral infection with ca. 10^7 CFU *C. difficile* spores (ATCC 43255).
[b] $n = 3$.

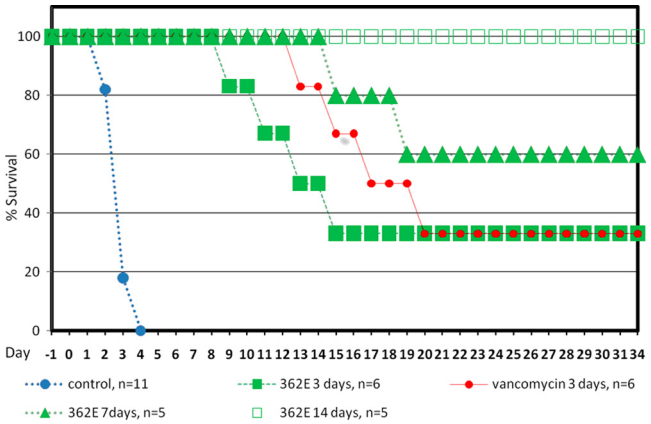

FIG 2 Acute cures and recurrences of CDAD in hamsters treated with 362E or vancomycin. Oral infection was on day 0 with 0.5×10^7 CFU of *C. difficile* strain ATCC 43255. The setup for the basic experimental protocol is described in the text. 362E (green squares) or vancomycin (red circles) was given twice daily at 50 mg/kg/dose by oral gavage from days 1 to 3, and, in separate groups, 362E was given by oral gavage from days 1 to 7 (green triangles) or 1 to 14 (open squares). Blue circles, untreated control animals.

In summary, our lead compound, 362E, is poorly absorbed from the GI tract and essentially nontoxic when given orally. These properties and the results presented in Tables 1 and 2 and Fig. 2 establish 362E as a worthy candidate for continued development as a new oral agent for the treatment of human CDAD.

Why develop another anti-*C. difficile* agent when there are other agents available, i.e., vancomycin, metronidazole, and most recently, fidaxomicin (1)? There are at least two significant reasons. First, 362E selectively hits pol IIIC, a target heretofore unexploited in *C. difficile* drug development. This property gives 362E strong potential for bypassing the resistance that will eventually emerge in *C. difficile* during the prolonged application of the agents in current use. Second, undesirable clinical issues frequently develop with prolonged use of any given therapy. For example, the widespread use of vancomycin, the current choice for therapy of severe CDAD, and metronidazole, the agent of choice for less severe disease, greatly increases the potential for patient shedding of both vancomycin-resistant enterococci and staphylococci (2, 3).

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REFERENCES

1. **Adis.** 2010. Fidaxomicin. Adis R&D profile. **10**:37–45. doi:10.2165/11537730.
2. **Al-Nassir WN, et al.** 2008. Both oral metronidazole and oral vancomycin promote persistent overgrowth of vancomycin-resistant enterococci dur-

ing treatment of *Clostridium difficile*-associated disease. Antimicrob. Agents Chemother. **52**:2403–2406.
3. **Al-Nassir WN, et al.** 2008. Comparison of clinical and microbiological response to treatment of *Clostridium difficile*-associated disease with metronidazole and vancomycin. Clin. Infect. Dis. **47**:56–62.
4. **Clinical and Laboratory Standards Institute.** 2007. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard, 7th ed. CLSI document M11-A7. CLSI, Wayne, PA.
5. **Johnson S, Gerding DN.** 1998. *Clostridium difficile*-associated diarrhea. Clin. Infect. Dis. **26**:1027–1036.
6. **Kelly CP, Pothoulakis C, LaMont JT.** 1994. *Clostridium difficile* colitis. N. Engl. J. Med. **330**:257–262.
7. **Kokkotou E, et al.** 2008. Comparative efficacies of rifaximin and vancomycin for treatment of *Clostridium difficile*-associated diarrhea and prevention of disease recurrence in hamsters. Antimicrob. Agents Chemother. **52**:1121–1126.
8. **McFarland LV.** 2008. Update on the changing epidemiology of *Clostridium difficile*-associated disease. Nat. Clin. Pract. Gastroenterol. Hepatol. **5**:40–48.
9. **Torti A, et al.** 2011. *Clostridium difficile* DNA polymerase IIIC: basis for activity of antibacterial compounds. Curr. Enzym. Inhib. **7**:147–153.
10. **Wright GE, et al.** 2005. Active site-directed inhibitors of replication-specific bacterial DNA polymerases. Bioorg. Med. Chem. Lett. **15**:729–732.
11. **Xu W-C, et al.** 2011. 7-Alkyl-N^2-substituted-3-deazaguanines. Synthesis, DNA polymerase III inhibition and antibacterial activity. Bioorg. Med. Chem. Lett. **21**:4197–4202.