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Comparative Efficacies of Rifaximin and Vancomycin for Treatment of Clostridium difficile-Associated Diarrhea and Prevention of Disease Recurrence in Hamsters⁷

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Clostridium difficile-associated colitis is an increasing cause of morbidity and mortality in hospitalized patients, with high relapse rates following conventional therapy. We sought to determine the efficacy of rifaximin, a novel nonabsorbed antibiotic, in the hamster model of C. difficile-associated diarrhea (CDAD). Hamsters received clindamycin subcutaneously and 24 h later were infected by gavage with one of two C. difficile strains: a reference strain (VPI 10463) and a current epidemic strain (BI17). Vancomycin (50 mg/kg of body weight) or rifaximin (100, 50, and 25 mg/kg) were then administered orally for 5 days beginning either on the same day as infection (prevention) or 24 h later (treatment). Therapeutic effects were assessed by weight gain, histology, and survival. We found that rifaximin was as effective as vancomycin in the prevention and treatment of colitis associated with the two C. difficile strains that we examined. There was no relapse after treatment with vancomycin or rifaximin in hamsters infected with the BI17 strain. Hamsters infected with the VPI 10463 strain and treated with rifaximin did not develop relapsing infection within a month of follow-up, whereas the majority of vancomycin-treated animals relapsed (0% versus 75%, respectively; P < 0.01). In conclusion, rifaximin was found to be an effective prophylactic and therapeutic agent for CDAD in hamsters and was not associated with disease recurrence. These findings, in conjunction with the pharmacokinetic and safety profiles of rifaximin, suggest that it is an attractive candidate for clinical use for CDAD.

Clostridium difficile is the most commonly identified cause of hospital-acquired infectious diarrhea in developed nations, accounting for up to 20% of cases of nosocomial diarrhea (4, 15). There are approximately 500,000 annual cases in the United States alone, with an estimated annual C. difficile-associated hospital cost of $3.2 billion (17, 26). The incidence of C. difficile-associated diarrhea (CDAD) has increased dramatically in the last 5 years, and serious outbreaks with high mortality have been reported (18, 21, 30, 31). Two of the current epidemic C. difficile strains, BI6 and BI17, belong to the BI/NAPI group according to restriction endonuclease analysis and pulsed-field gel electrophoresis, respectively, and to toxintype III according to restriction fragment length polymorphism analysis. They are characterized by the presence of the binary toxin CDT, by a deletion in the tcdC locus whose gene product negatively regulates the production of toxins A and B, and often by resistance to fluoroquinolones (21, 33). The initiating factor in the vast majority of cases is prior antibiotic therapy, which disrupts normal colonic flora allowing colonization by C. difficile (6) and production of two toxins, A and B, that cause intestinal inflammation (34). Almost any antibiotic can predispose to CDAD, but clindamycin, penicillin, cephalosporins, and fluoroquinolones are most commonly implicated (15, 18). Patients who acquire C. difficile infection may be asymptomatic carriers or may develop diarrhea, pseudomembranous colitis, or toxic megacolon. Mortality rates of 2 to 15% have been reported due to toxic megacolon, colonic perforation, sepsis, systemic inflammatory response syndrome, and requirement for emergency colectomy (1, 18, 21, 24, 27, 30).

The primary treatment for CDAD is administration of metronidazole or oral vancomycin (15). Prior to 2000, both metronidazole and vancomycin had reported efficacies of approximately 95% in CDAD (16). More recent data indicate that a failure to respond to metronidazole, the usual first-line agent, is now more common, raising concerns that the current treatment approach may be inadequate (3). Of further concern is the fact that relapse after treatment of initial infection is common, occurring in approximately 20% of cases overall and in some series in as many as 50% (29, 35). Recurrent CDAD may result from persistence of bacterial spores, reinfection from the environment, and failure to develop a protective immune response (17, 38). Although intermediate resistance of C. difficile strains to metronidazole and vancomycin has been reported, almost all episodes of recurrent CDAD result from strains susceptible to these antimicrobial agents and develop shortly after therapy has been completed (28).

Rifaximin, a nonabsorbed antibiotic when administered orally (8), is well tolerated and is almost completely excreted in the feces in its original form, making it ideally suited for use against C. difficile. It inhibits bacterial RNA synthesis, with activity against gram-positive and gram-negative aerobic and anaerobic bacteria (19). Rifaximin has been proven efficacious in preventing or treating traveler’s diarrhea, caused by diarrheagenic and enterotoxigenic strains of Escherichia coli (10)
and by Shigella (36). It also has excellent in vitro activity against C. difficile (19) and is associated with low rates of mutagenesis and resistance (19). In view of these characteristics, we sought to determine the effects of rifaximin at three different doses (25 mg/kg, 50 mg/kg, and 100 mg/kg of body weight) in a hamster model of CDAD, in which clindamycin administration, followed by exposure to C. difficile, leads to hemorrhagic cecitis similar to fulminant antibiotic-associated pseudomembranous colitis in humans (2, 9).

MATERIALS AND METHODS

Clindamycin-induced C. difficile colitis. Golden Syrian hamsters purchased from Charles River were housed in cages in groups of two with free access to chow (Purina 5000) and tap water. Hamsters were conditioned with a single subcutaneous injection of clindamycin phosphate (10 mg/kg) at day 1. At day 1, they received by gavage 10^5 CFU of toxigenic C. difficile strain VPI 10463 or BI17-6443. Hamsters received daily antibiotic treatments for days 1 to 5 in the prevention study and for days 2 to 6 in the treatment and relapse studies. Surviving hamsters were sacrificed on day 7 (prevention and treatment studies) or were monitored for disease relapse up to day 27.

Rifaximin and vancomycin prevent C. difficile-associated colitis. Hamsters (n = 10/group) were conditioned with clindamycin (day 0) and 24 h later (day 1) were infected with C. difficile (VPI 10463) and received the first dose of antibiotic or vehicle treatment which was continued daily for a total of five antibiotic doses (Fig. 1). Surviving animals were sacrificed at day 7. The survival rate in noninfected animals was 100%. All vehicle-treated animals developed severe colitis after infection with C. difficile and either died or were euthanized in a moribund state by day 3. In contrast, 80%, 70%, and 60% of animals receiving rifaximin treatment (100, 50, and 25 mg/kg, respectively) survived (Fig. 2A), indicating a dose-dependent effect of rifaximin (Table 1). Similar survival rates (70%) were from the recent epidemic that we tested in our model (12, 19). Moreover, the incidence of C. difficile mutants spontaneously resistant to rifaximin was found to be particularly low (<1 × 10^-6) (19), while 3 out of the 110 toxigenic clinical isolates were found to be resistant to rifaximin (12). Rifaximin (Salix Pharmaceuticals Inc.) was fully suspended in an aqueous solution of 0.1 M phosphate buffer (pH of 7.4) plus 4.5‰ sodium dodecyl sulfate. Vancomycin is also effective against toxigenic strains of C. difficile (MIC<sub>90</sub> of 1 mg/liter), and a dose of 50 mg/kg has previously been shown to be effective in the hamster model of CDAD (2, 23, 37). Hamsters (n = 10/group) were treated by gavage with daily doses of vancomycin (50 mg/kg) (Sigma), rifaximin (100, 50, and 25 mg/kg), or vehicle (4.5‰ sodium dodecyl sulfate in buffer) for a total of 5 doses. The administration of antibiotics was initiated at day 1 (prevention study) or at day 2 (treatment and relapse studies) as depicted in Fig. 1. The animals were weighed daily for 1 week and two to three times per week thereafter and observed twice per day for signs of morbidity or diarrhea. At the end of the observation period (day 7 or day 27), or at the time of death, the cecum was collected from each animal for histological evaluation of inflammation.

Histological examination. Hematoxylin and eosin-stained paraffin sections of the cecum were blindly evaluated by a gastrointestinal pathologist (M. O’Brien) and scored (0 to 3) for each of the following parameters associated with C. difficile colitis, as previously described by us (14): (i) epithelial damage, (ii) congestion and hemorrhage of the mucosa, and (iii) neutrophil infiltration. Histological analysis was performed in all animals included in the study, either at the time of their death due to C. difficile infection or at the end of the experiment. Statistical analysis. Data were analyzed by Kaplan-Meier survival analysis and the log rank test, analysis of variance with Bonferroni correction, Kruskal-Wallis nonparametric analysis, and a χ² test using the StatView statistical software program (Abacus Concepts, Berkeley, CA). Results are expressed as mean ± standard error unless otherwise indicated.

RESULTS

Rifaximin and vancomycin prevent C. difficile-associated colitis. Hamsters (n = 10/group) were conditioned with clindamycin (day 0) and 24 h later (day 1) were infected with C. difficile (VPI 10463) and received the first dose of antibiotic or vehicle treatment which was continued daily for a total of five antibiotic doses (Fig. 1). Surviving animals were sacrificed at day 7. The survival rate in noninfected animals was 100%. All vehicle-treated animals developed severe colitis after infection with C. difficile and either died or were euthanized in a moribund state by day 3. In contrast, 80%, 70%, and 60% of animals receiving rifaximin treatment (100, 50, and 25 mg/kg, respectively) survived (Fig. 2A), indicating a dose-dependent effect of rifaximin (Table 1).
Rifaximin prevents recurrence of C. difficile-associated colitis. We then studied a third cohort of hamsters with the intention of examining whether rifaximin and vancomycin were associated with differing rates of CDAD recurrence. Antibiotic treatment was administered on days 2 to 6 (Fig. 1). The 20 animals that survived their initial episode of C. difficile (VPI 10463) infection were maintained under observation for an additional 20 days after the termination of antibiotic treatment with rifaximin (either dose, n = 12) or vancomycin (n = 8) on day 6 (Fig. 1 and 4A). In a previous study with hamsters, we observed recurrence of C. difficile approximately 10 to 15 days after initial infection (2). As illustrated in Fig. 4A, 100% of rifaximin-treated hamsters (regardless of dosage level) survived to day 28 without recurrence of CDAD, while only 25% (two out of eight) of the vancomycin-treated animals survived the relapse (P < 0.01). After a period of weight loss immediately following C. difficile challenge, hamsters treated with 100 mg/kg of rifaximin recovered and started gaining weight, although at a lower rate than control animals (Fig. 4B). Overall, control hamsters gained 24.4% ± 1.2% and rifaximin-treated hamsters gained 16.7% ± 2.4% of their initial body weight (P < 0.01).

On histological examination (Fig. 4C), control animals had no lesions in the mucosal or submucosal areas while vehicle-treated animals exhibited extensive necrosis, congestion, and hemorrhage with reparative changes in residual crypts. Vancomycin-treated animals developed complete mucosal necrosis with hemorrhage, in contrast to rifaximin (100 mg/kg)-treated hamsters which had normal mucosa. As illustrated in Fig. 4D, control animals had uniformly normal cecal histology (0.0 ± 0 total histology score) and no lesions. All C. difficile-infected, vehicle-treated animals quickly developed severe colitis and had high histology scores (6.6 ± 0.5). The mean histological score among all the vancomycin-treated animals was 4.9 ± 1.0. Similar scores were observed in the hamsters treated with 25 mg/kg and 50 mg/kg of rifaximin, including the 2 out of 10 that did not survive, were significantly lower (1.6 ± 0.7) than those for each of the other four C. difficile-challenged groups (all groups, P < 0.001; and P of 0.02 compared to vancomycin). Histological appearance was completely normal in hamsters surviving to the end of the study.

Rifaximin and vancomycin are effective in preventing and treating infection with an epidemic strain of C. difficile (BI17) and preventing disease relapse. In addition to a reference toxigenic C. difficile strain (VPI 10463), we also examined the effectiveness of rifaximin in treatment of CDAD caused by
an epidemic strain (BI17-6443) (21). We conducted two studies, one for prevention of disease development and the other for treatment (for rifaximin and vancomycin treatments, $n_{/H11005}$10/group; and for vehicle, $n_{/H11005}$8/group); their design was as described in the legend for Fig. 1. Both cohorts were monitored for one month, to assess rates of recurrent infection. All mice treated with either rifaximin (100 mg/kg) or vancomycin (50 mg/kg) survived the acute infection with this hypervirulent strain. Moreover, we did not observe any disease relapse with this particular strain of *C. difficile* in antibiotic-treated hamsters during the follow-up period.

**DISCUSSION**

We report here that rifaximin was equivalent to vancomycin in prevention and treatment of weight loss, histological inflammation, and fatal CDAD in hamsters caused by two different *C. difficile* strains. However, hamsters treated effectively with rifaximin for acute infection with the *C. difficile* strain VPI 10463 did not develop recurrent fatal cecitis after discontinuation of therapy, whereas the majority of vancomycin-treated animals relapsed (0% versus 75%, respectively; $P < 0.01$). In humans, the standard management of CDAD is discontinuation of the precipitating antibiotic(s) and treatment with metronidazole or vancomycin (20). However, one recent study reported overall efficacy rates of only 50% with metronidazole, with 22% of patients remaining symptomatic despite treatment (25). In the present study, the overall efficacy in treating initial infection in hamsters, including data from all three cohorts, was 87% for rifaximin (100 mg/kg) (bottom left): histological normal cecal mucosa. Vancomycin (50 mg/kg) (bottom right): histological normal cecal mucosa. (C) Hematoxylin and eosin-stained histological biopsies of cecal tissue from all hamsters enrolled in the study were blindly evaluated by an experienced pathologist and scored (0 to 3) for (i) epithelial damage, (ii) congestion and hemorrhage of the mucosa, and (iii) neutrophil infiltration. Histological score represents the sum of the above scores. **, $P < 0.01$ compared to vehicle treatment.
epidemics of CDAD have been associated with increased relapse rates (5). To our knowledge, studies of recurrent CDAD in hamsters have not been reported previously for a BI strain, and our findings may reflect interspecies differences in disease manifestations and severity between humans and hamsters. The most-effective dose of rifaximin used in this study (100 mg/kg) is almost 10-fold higher than the dosage used in patients with CDAD (400 to 800 mg daily, in two to three divided doses) (11, 13). The dose of vancomycin used in hamsters (50 mg/kg) is also higher than the one used in humans (500 mg) (2, 23, 37); therefore, it is plausible that it results in a greater disturbance of the normal flora and an increased risk for relapse in this model. However, studies in humans do not support this concern, since they report high-dose vancomycin to be at least equivalent to low-dose vancomycin in preventing relapse (22). Moreover, the same study concluded that the duration of vancomycin therapy, more than the dose per se, is the most important determinant of risk for relapse (22).

The management of recurrent CDAD remains problematic, and in addition to repeat courses of vancomycin, therapies such as probiotics, which restore the normal flora, agents that block toxin A binding, such as cholestyramine, and immunotherapy with anti-toxin A antibodies have been applied (20). Rifaximin treatment of the initial infection might prove to be beneficial for preventing relapse in clinical practice. Alternatively, it could be used to treat the first relapse and thus reduce the risk for subsequent episodes.

Due to minimal systemic absorption (<1%) (8), rifaximin was found in randomized clinical trials to be a safe drug, with adverse effects not different from those of placebo (10, 32). It has also been reported that rifaximin had minimal effects in altering the intestinal microflora with respect to coliforms and enterococci (7, 10). The ability of rifaximin to preserve elements of the colonic flora while eradicating C. difficile may be important in restoring colonization resistance and provides another possible mechanism for the absence of recurrent CDAD after rifaximin therapy. Studies of bacterial resistance to rifaximin have demonstrated that C. difficile has a very low incidence of mutants spontaneously resistant to rifaximin (19). However, among 110 toxigenic clinical isolates evaluated, three of them (two from Argentina in 1998 and one from Chicago in 1995) were found to be resistant to rifaximin in vitro (12).

In conclusion, rifaximin is effective for prevention and treatment of fulminant C. difficile-associated colitis in clindamycin-treated hamsters. Our major finding was that, compared to vancomycin, rifaximin was associated with significantly lower rates of recurrent CDAD after completion of therapy for the initial infection with the VPI 10463 strain of C. difficile. Lack of systemic absorption and a good safety profile make rifaximin an attractive candidate for use in the treatment of CDAD in humans. Indeed, while this paper was under revision, the first report of rifaximin preventing recurrence of C. difficile infection in seven out of eight women with a history of multiple episodes of CDAD was published (13). These data indicate the
need for prospective controlled trials of rifaximin both for primary therapy and for secondary prevention of CDAD.

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