REVIEW

Prevention and treatment of *Clostridium difficile* associated diarrhea by reconstitution of the microbiota

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ABSTRACT

This review summarizes the latest advances in treating and preventing *Clostridium difficile* infection (CDI), the most common infectious disease cause of nosocomial diarrhea in adults in developed countries. As customary antibiotic therapies against *C. difficile*, metronidazole and vancomycin, are broad spectrum, they affect greatly the gut microbiota, which result in very high recurrence rates. Therefore, new strategies are researched intensively. New therapies focus on limiting further destruction of the gut microbiota or restoring the microbiota to its pre-deconstructed state. These include new antibiotics, such as fidaxomicin, which demonstrates reduced CDI recurrences, among other new drugs, biotherapeutic strategies, mainly fecal microbiota transplantation but also competitive inhibition with non-toxigenic strains of *C. difficile*, and finally, monoclonal antibodies against *C. difficile* toxins which offer protection against recurrences.

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Introduction

Clostridium difficile (*C. difficile*) is a Gram-positive, anaerobic spore producing rod. *C. difficile* infection (CDI) is the most common infectious nosocomial diarrhea among adults in developed countries. Increasing morbidity and mortality has been reported recently, with recent estimated in the United States of 500,000 infections annually.¹ CDI prolongs hospitalization, raises hospital expenses, and recurs in one fifth of the patients.²

Exposure to *C. difficile* alone will not cause CDI. Interruption of the intestine microbiota by antibiotics creates a favorable environment for *C. difficile* spores to flourish, inhabit the colon and release toxins which cause symptomatic CDI. These microbiota disruptions are well-documented in adults using 16S ribosomal RNA gene amplification and have demonstrated the changes that result from antimicrobial exposure.³ Patients with recurrent CDI have markedly altered gut microbiota in terms of both bacterial diversity and numbers. Reconstitution of the host's microbiota protects from recurrence of CDI.⁴ The host's immune system is also of great importance, for example, the absence of antibodies against *C. difficile* toxin or the lack of functioning humoral immune system, will lead to the development of recurrence of CDI.⁵

A thorough understanding of the pathophysiology of the disease lead to the development of new, promising strategies in treating and preventing CDI.

Antibiotics

Metronidazole and vancomycin

For several decades, metronidazole and oral vancomycin remain the first line therapy in many countries. Metronidazole

inhibits nucleic acid synthesis by disrupting the DNA of microbial cells. Vancomycin is a glycopeptide that prevents cell-wall biosynthesis of bacteria. For severe disease or second recurrence oral vancomycin is the preferred agent. This practice is based mainly on a small randomized controlled trial (RCT) published in 2007 that randomized 150 patients and demonstrated that vancomycin lead to higher clinical cure rates than metronidazole in severe disease, with no difference in mild-moderate disease.⁶ Both metronidazole and vancomycin have a broad coverage spectrum, which suppress the intestinal microbiota, and are therefore associated with unacceptably high CDI recurrence rates. Thus intensive research is taking place, seeking microbiota-sparing antibiotics that, at least in part, overcome this limitation.⁷

Fidaxomicin

Fidaxomicin was approved recently for the treatment of CDI. It is a macrocyclic oral antibiotic that inhibits bacterial nucleic acid synthesis by inhibiting bacterial RNA polymerase. This is a bactericidal, narrow spectrum, highly selective against *C. difficile* (eight times more bactericidal than vancomycin). It has reduced activity against several enteric commensal bacterial species, especially that confer colonization resistance against *C. difficile* (as *Bacteroides* spp. other anaerobic Gram-negative bacilli and other *Clostridium* spp.). It is not absorbed systemically and has a minimal effect on the gut's microbiota due to its mechanism. A RCT published by Louie et al in 2011 compared treatment of 10 days with vancomycin to fidaxomicin in 596 adults with a first or second episode of CDI, showed noninferiority of fidaxomicin in efficacy and safety, with statistically

significant less recurrences in the fidaxomicin arm (15% versus 25% with vancomycin, $p = 0.005$).⁸ Another similar RCT conducted in Europe and North America showed similar results⁹ and a combined analysis of the two trials demonstrated a significant advantage for fidaxomicin in prevention of recurrences including in a subgroup of patients who required concomitant antibiotics during CDI (17% in fidaxomicin arm versus 29% in vancomycin, $p = 0.048$).¹⁰ A recent RCT published in *Lancet Infect Dis*, randomized 362 patients 60 years and older, to receive extended-pulsed fidaxomicin (200 mg oral tablets, twice daily on days 1–5, then once daily on alternate days on days 7–25) or vancomycin (regular dose for 10 days). Extended-pulsed fidaxomicin was superior to standard-dose vancomycin in achieving sustained clinical cure of CDI 30 days after end of treatment (124/177 (70%) in fidaxomicin arm versus 106/179 (59%) in vancomycin arm).¹¹ Disease-free survival was higher in the extended-pulsed fidaxomicin group. In addition, recurrence rate of CDI in the extended-pulsed fidaxomicin arm was the lowest reported in previous studies, and most importantly, it has been shown that the gut microbiota diversity increased to a higher degree in extended-pulsed fidaxomicin-treated patients than in vancomycin-treated patients, indicating that extended-pulsed fidaxomicin therapy enhanced gut microbiota resilience significantly better than vancomycin, making fidaxomicin a microbiota-preserving anti-*C. difficile* agent.^{11,12}

Antibiotics in the horizon

Cadazolid

An oxazolidinone antibiotic with bactericidal activity, similar in structure to linezolid, inhibits protein synthesis, *C. difficile* sporulation and toxin formation, although it is not bactericidal. In contrast to metronidazole and vancomycin, cadazolid strongly inhibited de novo toxin A and B formation in stationary-phase cultures of toxigenic *C. difficile*, and also inhibited *C. difficile* spore formation. In a human gut model, it has been shown that cadazolid has a very limited impact on the indigenous gut microbiota. It has a minimal activity against other Gram-positive pathogens, including *Staphylococcus aureus* and enterococci, but has almost no activity against most Gram-negative bacteria, including (notably) the *Bacteroides* group.¹³

In a RCT phase II (2015) comparing three different doses of oral cadazolid to oral vancomycin for first episode or first recurrence of CDI, cadazolid demonstrated noninferiority in both efficacy and safety comparing to vancomycin, in all doses. This trial showed a trend for less recurrences with cadazolid but this did not reach statistical significance.¹⁴

Ridnilazole

A novel antibiotic that does not work in the classical mechanisms, it inhibits DNA synthesis, has high potency against *C. difficile* comparing to other Gram-negative and Gram-positive aerobic and anaerobic intestinal bacteria, and is active only in digestive system. A small phase III RCT (2017) comparing oral ridnilazole to oral vancomycin included 100 patients randomized 1:1, and showed ridnilazole was superior to vancomycin for sustained clinical cure within 30 days [24/36 (66.7%)

ridnilazole compared to 14/33 (42.4%) vancomycin, $P < 0.05$], with similar efficacy.¹⁵ Sustained clinical cure, in this study reflected both initial clinical cure along with recurrence rate. Therefore, the superiority of ridnilazole compared with vancomycin was due to the lower recurrence rate in the ridnilazole group, probably thanks the gut-microbiota preserving capabilities of this drug.

Surotomycin

An oral lipopeptide antibiotic, similar in structure to daptomycin, disrupts the bacterial cell membrane. It is selective against *C. difficile*, therefore has a minimal effect on the gut microbiota. A phase III RCT (2017) comparing oral surotomycin to oral vancomycin included 577 patients showed surotomycin was non inferior to vancomycin for clinical cure at end of treatment, but did not reduce recurrences.¹⁶

Biotherapeutics

Biotherapeutics strategies gaining considerable interest, especially fecal microbiota transplantation (FMT) for treating patients with more than one recurrence. Antibiotic disruption of the intestinal microbiota is the postulated mechanism that enables ingested *C. difficile* spores to vegetate, colonize the gut, produce toxins and cause colitis. The idea of biotherapies is to try to rehabilitate the intestinal microbiota. The first RCT was published in the *NEJM* in 2013 included 43 patients with 1–9 recurrences that were randomized to FMT (per nasogastric tube) or standard vancomycin treatment (14 days) or vancomycin treatment (14 days) with gastrointestinal lavage. The study was terminated prematurely because of superiority of the study arm with 13/16 (81%) of patients in the FMT arm recovering after one donor-feces infusion, comparing to 4/13 (31%) in the vancomycin group ($p = 0.003$), and 3/13 (23%) in the third group ($p = 0.008$). In patients who failed first FMT, a second FMT was performed raising response rate in this arm to 94%.¹⁷ A more recent comparison was done in Feb 2017 by Hota et al. who compared FMT in patients with more than one recurrence to tapering down of vancomycin. This small RCT included 30 patients with acute CDI and more than one recurrence, they all received 14 days of standard vancomycin treatment and then randomized to FMT (enema) versus tapering down of vancomycin over 6 weeks. The study was terminated early because of futility analysis, when no difference was demonstrated between the study groups in response or recurrence rate.¹⁸ Apart from patients with recurrences, small non-randomized trials showed partial success with great variability treating patients with severe refractory disease or first severe episode of CDI with FMT. A recently published long term follow up of 22 months after 137 patients receiving FMT, 82% (113/137) of patients had no recurrence. Patients with recurrence had more antibiotic exposure after transplant, underscoring the need for thoughtful antibiotic use and for prophylactic microbiome enrichment and restoring the intestinal microbiota to reduce recurrence.¹⁹

A few trials demonstrated that freezing stool does not result in loss of effectiveness, therefore stool banks were raised for donating stool worldwide. Open questions remain regarding

the preferred administration route for FMT (enema, nasogastric tube, endoscopy, colonoscopy), regarding safety of the different methods (studies included small numbers of patients leaving this question open) and the balance between cost and benefit. Today there is commercially available for purchase frozen FMT and a growing interest in oral capsules that are being studied intensively.^{20–22}

Replacing toxigenic strain of *Clostridium difficile* by nontoxigenic *Clostridium difficile* strain

The idea is to try to replace the toxigenic strain of *C. difficile* by nontoxigenic *C. difficile* strain by oral intake of the nontoxigenic *C. difficile* strain M3 type (NTCD-M3). This strain does not cause CDI and results only in asymptomatic colonization. The mechanism is competitive inhibition. In a RCT published in 2015 by Gerding et al, 168 patients with a primary CDI or first recurrence that finished treatment with metronidazole or vancomycin, were randomized to placebo or NTCD-M3 for 7–14 days in different doses. The study group demonstrated similar safety with statistically lower recurrence rates versus placebo (11% in study group versus 30% in placebo group, $p = 0.006$).²³

Immunological therapies

The relative biologic importance of toxins A and B in *C. difficile* infection is controversial, and may be host species dependent. In rodents, neutralization of both toxins seems to be necessary for maximal protection from CDI, but neutralization of toxin B alone appears to be sufficient in piglets. In humans, the level of IgG antibodies against toxin A or toxin B have been correlated with protection against primary and recurrent CDI. Levels of IgG antibodies against *C. difficile* toxins A and B are also higher in asymptomatic adults who carry *C. difficile*, than in adults who develop CDI.⁵ This suggests that the humoral immune system and antibodies against *C. difficile* toxins protect from CDI. Two RCTs were published in the NEJM including 2,665 patients with a first episode or recurrent CDI. Participants received monoclonal antibodies against toxin A, B or placebo. The study arm of toxin A was stopped prematurely for showing inferiority and more side effects in interim analysis. Final results showed lower recurrence rates for patients receiving antibodies against toxin B comparing to placebo, in both studies, with equal cure rate (first study, for recurrence: 37/386, 17% toxin B Ab versus 109/395, 28% placebo, $p < 0.001$; second study, for recurrence: 61/383, 16% toxin B Ab versus 97/378, 28% placebo, $p < 0.001$).²⁴

Vaccines for CDI

Vaccines for *C. difficile* have also been studied and developed, and may provide another alternative to antibiotic treatment, with hope for sustained long-term protection against future CDI episodes. There are three vaccine candidates undergoing phase II and III clinical evaluation for CDI prevention, all given IM in three doses.²⁵ The first, by Sanofi Pasteur is a toxoid vaccine, the antigen is formalin-inactivated toxins A and B from VPI 10463, in phase III clinical trials.²⁶ The second, by Valneva Austriauses recombinant fusion protein of toxin A and B

binding regions.²⁷ The third, by Pfizer, is a recombinant vaccine, genetically modified and chemically treated.²⁸ These last two are in phase II clinical trials. There are no clinical trials yet assessing the developing orally administered mucosal vaccines, but these are highly attractive. Assuming an effective vaccine is developed, many questions remain to be answered regarding these vaccines, for example, what population should be targeted? What is the optimal age for immunization and what is the duration of protection the vaccines give.

Summary and recommendations

In summary, CDI remains a great challenge for treating and preventing. Many treatment strategies are being researched, each has pros and cons, maybe combining different strategies, exploiting the cons from each strategy, will lead to the best clinical results. Future studies will focus mainly on prevention and microbiota reconstruction.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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