Addressing a *difficile* problem

By Kai-Jye Lou, Staff Writer

Researchers in Ireland and Canada have identified a bacterial peptide with narrow-spectrum antimicrobial activity against *Clostridium difficile*.¹ The peptide, which is licensed to Alimentary Health Ltd., could reduce the risk of disease recurrence compared with that of broad-spectrum antibiotic treatment because it spares the normal gut flora that helps limit *C. difficile* growth.

C. difficile is a Gram-positive opportunistic bacterium that can cause gastrointestinal conditions such as diarrhea and pseudomembranous colitis after a patient has been treated for unrelated bacterial infections.

Antibiotics can unbalance normal gut flora, thus creating an environment that could be conducive to *C. difficile* growth. Even if the antibiotic affects *C. difficile* growth, the bacterium produces resistant spores, which can germinate and cause disease.²

C. difficile infections are routinely treated with metronidazole or oral Vancocin (vancomycin), but these drugs also wipe out the gut flora that keeps *C. difficile* in check. *C. difficile* recurs in about 20% of patients treated with these antibiotics, and the rate increases with subsequent recurrences.³

Vancocin, a broad-spectrum tricyclic glycopeptide antibiotic, is marketed by ViroPharma Inc. in the U.S. and by Iroko Pharmaceuticals LLC in Canada.

"Recovery of normal gut flora is important for recovery from *C. difficile*-associated disease, but the use of broad-spectrum antibiotics can delay this process," noted Colin Hill, a professor of microbial food safety in the Alimentary Pharmabiotic Centre at University College Cork (UCC).

Given the importance of normal gut flora in controlling *C. difficile* growth, Hill and colleagues at the Teagasc Moorepark Food Research Centre analyzed the very bacterial populations that keep *C. difficile* at bay during normal conditions, with the goal of finding a compound that could specifically eliminate this organism.

"We screened over 30,000 isolates from human fecal samples for specific activity against *C. difficile*. Of those, only one had the spectrum of activity that we were looking for," said Hill.

The single isolate contained a strain of *Bacillus thuringiensis* that produced a bacteriocin—an antimicrobial peptide produced by bacteria—with narrow-spectrum activity against *C. difficile*. The bacteriocin, designated thuricin CD, consists of two distinct peptide components: Trn- α and Trn- β .

In a panel of bacteria, thuricin CD had antimicrobial activity against multiple types of *Clostridium*, including *C. difficile*. It also was active against *Listeria monocytogenes*, a Gram-positive, food-borne pathogen. Thuricin CD had little or no activity against other pathogenic or commensal bacterial strains.

In an *ex vivo* human distal colon model, thuricin CD significantly reduced *C. difficile* viability compared with that seen in untreated controls ($p < 0.001$).

Results were published in *The Proceedings of the National Academy of Sciences*. Hill co-led the research with R. Paul Ross, head of food research at Teagasc and a principal investigator at UCC's Alimentary Pharmabiotic Centre.

Other researchers are looking at ways to attack *C. difficile* without affecting normal gut flora.

Optimer Pharmaceuticals Inc. expects to submit an NDA next half for fidaxomicin, a macrocyclic narrow-spectrum antibiotic to treat *C. difficile* infections. In February, the company reported Phase III data showing that fidaxomicin was not inferior to Vancocin on the primary endpoint of clinical cure rate in the population studied per their protocol. Fidaxomicin also had a significantly lower rate of recurrence and a significantly higher global cure rate, both secondary endpoints, than did Vancocin.

In an e-mail to SciBX, Jared Silverman and Steven Gilman of Cubist

Pharmaceuticals Inc. noted that "a very narrow spectrum agent has been considered the best approach for *C. difficile*." Silverman is senior director of *in vitro* biology, and Gilman is CSO and SVP of discovery and non-clinical development, at the company.

They added that the fidaxomicin data "suggest that having a narrower spectrum than Vancocin does indeed reduce relapse rates."

Cubist's CB-183,315, an oral antibacterial lipopeptide, is in Phase II testing to treat *C. difficile*-associated diarrhea (CDAD).

Silverman and Gilman also noted it will be important to obtain "more quantitative values of drug concentration" for thuricin CD. That's particularly important when evaluating *C. difficile* treatments, they said, because "drug concentrations in the GI tract are typically very high." Thus, a compound's inhibitory effects against off-target bacteria can be accentuated in the gut microenvironment.

Despite this potential limitation, both Silverman and Gilman reiterated that thuricin CD is "a potentially interesting compound that definitely merits further investigation."

Delivering the goods

The key steps going forward are to develop a way to deliver thuricin CD and to produce the bacteriocin in sufficient quantities.

"We have since developed an *in vitro* colon model and shown that thuricin actively kills *C. difficile*, so the compound should work *in vivo*," Hill told SciBX. "But we will need to develop a way to deliver it."

Because thuricin CD is a peptide, it will need to be encapsulated before it can be given orally, otherwise "the naked peptide will get chewed up by

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digestive processes before it can get to the gut,” he said.

Yoshi Ichikawa, executive director of IP and discovery at Optimer, said it also will be important to develop a way to efficiently produce enough of the thuricin peptides from *B. thuringiensis*. He said thuricin CD seems to be a relatively large peptide with post-translational modifications, and as a result could pose production challenges.

He also wanted to see a study comparing thuricin CD with oral Vancocin in animals infected with *C. difficile*.

Teagasc and UCC hold a patent covering thuricin CD and its use in treating *C. difficile* infections. The patent has been licensed to Alimentary, which is developing therapeutics and healthcare products derived from commensal gut bacteria for gastrointestinal inflammatory conditions such as irritable bowel syndrome (IBS), Crohn's disease and ulcerative colitis (UC), and infectious diseases including *C. difficile*.

Alimentary did not respond to requests for comment.

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COMPANIES AND INSTITUTIONS MENTIONED

Alimentary Health Ltd., Cork, Ireland
Cubist Pharmaceuticals Inc. (NASDAQ:CBST), Lexington, Mass.
Iroko Pharmaceuticals LLC, Philadelphia, Pa.
Optimer Pharmaceuticals Inc. (NASDAQ:OPTR), San Diego, Calif.
Teagasc Moorepark Food Research Centre, Fermoy, Ireland
University College Cork, Cork, Ireland
ViroPharma Inc. (NASDAQ:VPHM), Exton, Pa.