

***Clostridioides difficile* toxins inhibit the functions of colonic ATP-binding cassette transporters**

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Background: *Clostridioides difficile* (*C. difficile*) is an enteric pathogen that is known for its ability to produce toxins. *C. difficile* can produce three types of toxins: TcdA, TcdB, and binary toxin. The effects of these toxins on the body can be devastating as they can cause unremitting diarrhea, toxic megacolon, and even death. As a result, research is needed to better understand how *C. difficile* causes disease. ATP-binding cassette (ABC) transporters are membrane proteins that regulate the movement of diverse molecules across the intestinal epithelium. These transporters contribute to epithelial homeostasis by controlling the absorption and efflux of metabolites, lipids, and potentially harmful compounds. However, the effects of *C. difficile* toxins on intestinal ABC transporters remain poorly understood. This study aimed to elucidate the interaction between *C. difficile* toxins and their ability to disrupt the functions of important ATP-binding cassette transporters. **Methods & Results:** Analysis of RNA-sequencing (RNAseq) data from *C. difficile* infected mice revealed decreased expression of colonic ABCB1, ABCC2, and ABCG2 compared to vehicle control mice. Immunostaining of CDI mouse colonic tissue for ABCB1 levels confirmed decreased ABCB1 levels in CDI mice at the peak of infection compared to healthy mice. We also examined RNAseq data from mice receiving a rectal administration of TcdA or TcdB. We found that after 16 hrs of toxin exposure, the colonic cells had significantly reduced ABCB1 expression compared to mock treated mice. These data suggest that both TcdA and TcdB can reduce ABC transporters. To assess if *C. difficile* toxins could directly impact these ABC transporters, we treated human colonic T84 cells with *C. difficile* toxins TcdA, TcdB, or binary toxin and examined transcription by RNAseq. Excitingly, we found that all toxins could reduce ABCB1. To analyze the function of ABCB1, we performed a Rhodamine 123 assay in T84 cells. We found that the administration of *C. difficile* toxins significantly reduced ABCB1's ability to efflux Rhodamine 123; suggesting a loss of ABCB1 function in response to *C. difficile* toxins. **Conclusions:** These data provide the first evidence of *C. difficile* toxin-mediated inhibition of ATP-binding cassette transporters such as ABCB1. We speculate that disruption of these transporters may contribute to epithelial dysfunction and disease severity during *C. difficile* infection.