The Effect of *Clostridium difficile* toxin on copper homeostasis in neutrophils

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Introduction: *Clostridium difficile* infections are a possible complication of antibiotic treatment regimens as gut microbiome biodiversity is reduced, allowing *C. difficile* to overgrow and produce toxins, leading to diarrhea and potentially pseudomembranous colitis, which can be life-threatening. Neutrophils are white blood cells that are part of the body's innate immune response to infections, using cytoskeletal rearrangement to transmigrate across epithelial layers to reach sites of infection and form structures that trap pathogens. Previous studies have shown that *C. difficile* toxin B (CDT B) can disrupt cytoskeletal rearrangement, affecting neutrophil migration. While this mechanism is not completely understood, one hypothesis proposes that the toxin disrupts a neutrophil's copper homeostasis. This project aims to investigate whether CDT B influences copper uptake by neutrophils by measuring the expression of surface copper transporter protein 1 (CTR1) and copper-transporting ATPase 2 (ATP7B) via flow cytometry.

Methods: Samples were prepared from neutrophils extracted from whole blood obtained from human donors. Post-extraction, the cells were separated into four solutions that had a different concentration of Cu^{2+} (0 μM, 5 μM, 10 μM, 25 μM). After incubation, the samples were stained with an antibody that binds either CTR1 or ATP7B and a viability stain (7-Aminoactinomycin D, 7-AAD). After staining, flow cytometry was performed to capture the fluorescence of the stained surface receptors. Mean fluorescence intensity (MFI) was then calculated after gating for live neutrophils with either CTR1 or ATP7B stain. A one-way ANOVA and post-hoc Tukey tests were performed to assess any significant differences in MFI between samples.

Results: In samples staining for CTR1, one-way ANOVA analysis showed that copper concentration had a significant impact on MFI, $F(3, 26852) = 27.80$, $p < 0.001$. Post-hoc Tukey tests showed that MFI was significantly greater in each sample (5, 10, 25 μM) when compared to the 0 μM sample, but there was no significant difference in MFI between the 10 μM and 25 μM samples. A similar trend was seen in the data generated from samples stained for ATP7B, where the ANOVA showed that copper concentration had a significant impact on MFI, $F(3, 20460) = 26.06$, $p < 0.0001$. Post-hoc Tukey tests showed that there was no significant difference in MFI between 0 μM and 5 μM, but differences between 5 μM, 10 μM, and 25 μM were significant. Conclusion: There is a significant increase in MFI when neutrophils are exposed to increasing levels of copper. This shows that the surface expression of CTR1 and Copper-Transporting ATPase2 in neutrophils increases when exposed to increasing concentrations of copper. With this relationship established, further experiments are needed to see how the expression of these copper transporters changes when the cells are exposed to non-cytotoxic levels of CDT B.

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