Indomethacin Decreases Survival in *Clostridioides difficile* Infection in an Insect Model, *Galleria mellonella*

Poster# 2

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BACKGROUND

- *Clostridioides difficile* infection (CDI) is an urgent public health threat worldwide.
- Previous experimental rodent studies have shown that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with enhanced severity of CDI (1).
- Based on previous studies, it was suggested that NSAIDs enhanced severity of CDI via altering prostaglandin (PG) metabolism by inhibiting cyclooxygenase (COX) enzymes, resulting in changes in proinflammatory transcriptional and protein profile, and perturbation in epithelial cell junctions (2).
- These effects were paralleled by increased recruitment of intestinal neutrophils and CD4+ cells as well as significant alteration in the gut microbiota.
- Together, these data implicate NSAIDs cause disruption in the protective COX-mediated PG production during CDI, resulting in altered epithelial integrity and associated immune responses.
- The invertebrate model *G. mellonella* has become an attractive alternative to other *in vivo* models in infectious diseases related research, including bacterial and fungal virulence, viral infections, and antimicrobial screening and testing.
- This popularity is attributed to its low cost, short life cycle, simple handing, and lack of ethical constraints.
- This effect of NSAIDs has never been investigated in a simple in vivo model such as *G. mellonella.*

OBJECTIVE

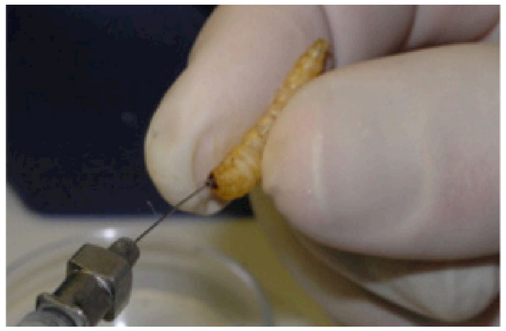
- To assess the feasibility of using *G. mellonella* as a surrogate insect model to evaluate the effect of NSAIDs on CDI severity and survival

METHODS

Larval inoculation and treatment:

- Larvae were gavaged with 1x10^{6-8} CFU of two *C. difficile* ribotype 027 strains (R20291, CD 196) and one ribotype 014-020 strain (MT-5313)
- Larvae were pretreated with indomethacin (5 µg/larva) via gavage 24 hours prior to *C. difficile* inoculation
- The larvae were kept at 37°C post-infection and monitored daily for 120 hours for survival.
- Larvae were assigned into the following arms and experiments were repeated in duplicate:
  1. Negative control (PBS only), n = 60
  2. Positive control (*C. difficile* only; n=20 for each strain), n = 60
  3. Indomethacin+*C. difficile* inoculation (n = 20 for each strain), n = 60

Figure 1. Force feeding (gavaging) of *G. mellonella*



Determination of *C. difficile* count in *G. mellonella* larvae:

- Two larvae from each group were randomly selected, hemolymph was extracted and serially diluted, *C. difficile* were counted by using most probable number (MPN) method

Determination of hemocyte density in *G. mellonella* larvae:

- Two larvae from each group were randomly selected, hemolymph was extracted and serially diluted, hemocytes were counted under light microscope

RESULTS

Figure 2. Larvae overall survival (n=180)

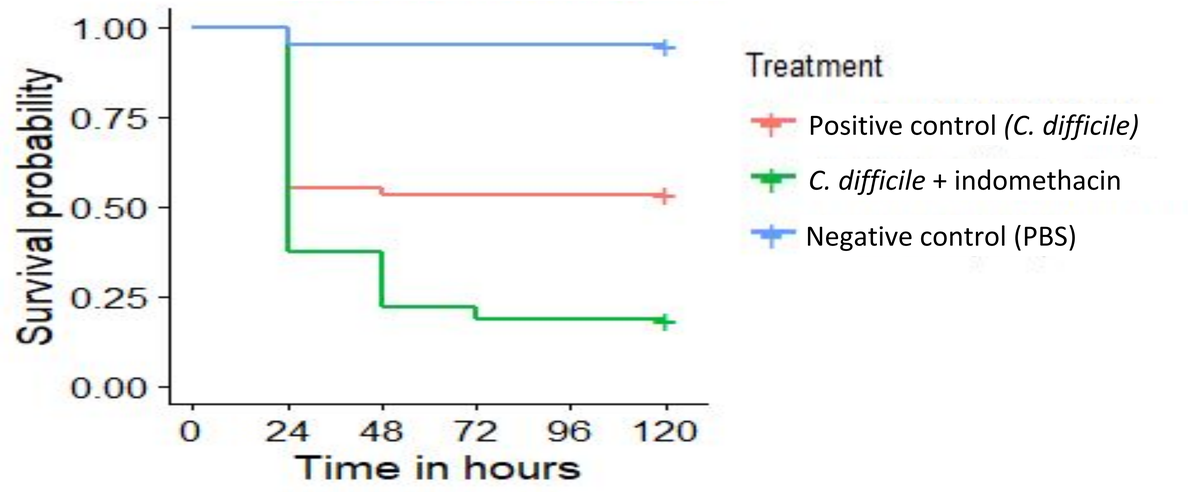


Figure 3. Mean hemocyte density per larva

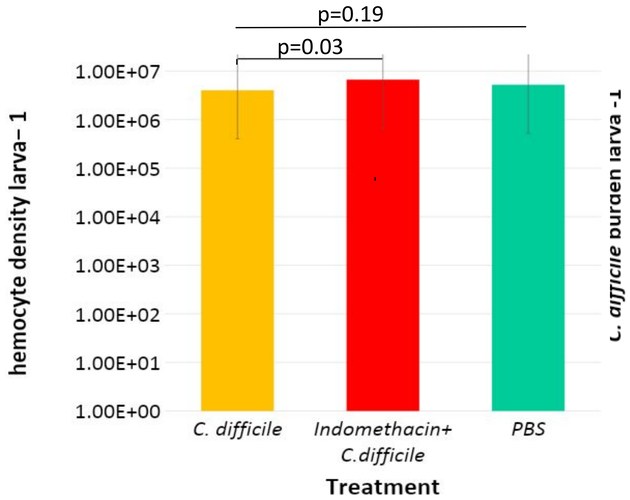
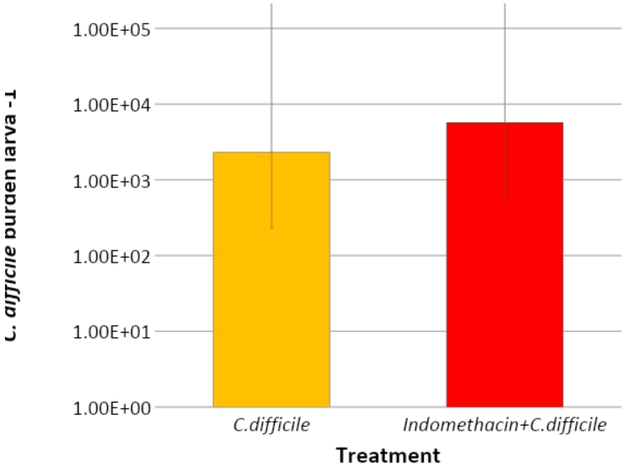


Figure 4. Mean *C. difficile* burden per larva



CONCLUSIONS

- ***G. mellonella* larvae display increased mortality from *C. difficile* infection when force fed indomethacin, similar to what has been demonstrated in rodent models.**
- **We hypothesize this may be mediated through exacerbation of immune responses in *G. mellonella* larvae, but further studies are needed.**

REFERENCES

1. Muñoz-miralles J, Trindade BC, Castro-córdova P, et al. Indomethacin increases severity of *Clostridium difficile* infection in mouse model. *Future Microbiol*. 2018;13:1271-1281. https://doi.org/10.2217/fmb-2017-0311.
2. Maseda D, Zackular JP, Trindade B, et al. Nonsteroidal anti-inflammatory drugs alter the microbiota and exacerbate colitis while dysregulating the inflammatory response. *mBio*. 2019;10(1). https://doi/10.1128/mBio.02282-18.