

ABSTRACT

Chemotherapy has been shown to disrupt intestinal bacterial populations that can lead to complications such as diarrhea, bacteremia, and septicemia. This research seeks to determine the effect of the common chemotherapeutic agent, 5-fluorouracil (5-FU), on the survival rate of the normal intestinal organisms *Bifidobacteria sp*, *Lactobacillus sp*, *Bacteroides sp*, and *Escherichia coli* in vitro. Initially, test organisms were grown in tubes and exposed to four different 5-FU doses that were estimated to be within the physiological range in the intestine following chemotherapeutic infusion therapy. Growth inhibition was not observed in any of the bacterial species tested. When a more sensitive instrument became available for testing, the experiment was repeated on a smaller scale in 96 microwell plates. Significant growth inhibition proportional to the drug dose was observed only in *Escherichia coli*. Though decreased populations of *Bifidobacteria spp*, *Lactobacillus spp*, and *Bacteroides spp* and increased populations of *Escherichia spp* have been observed in patients taking 5-FU, the opposite effect has been shown by this study. 5-FU inhibited the growth of *Escherichia coli*, but the growth of *Bifidobacteria sp*, *Lactobacillus sp* and *Bacteroides sp* did not show similar inhibition.

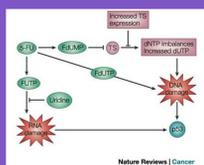
HUMAN MICROBIOME

The human microbiome is made up of commensal microorganisms that inhabit the human body. The highest concentration of microorganisms is in the intestine. Many studies have shown association between changes in the human microbiome and several disease states such as IBS and chemotherapy-induced gastrointestinal mucositis.

5-FLUOROURACIL (5-FU)

Chemotherapeutic drug used in the treatment of many different cancers. Studies have shown decreases in *Bifidobacteria* and *Lactobacillus* populations in patients who have been administered 5-FU.

Mechanism of Action: DNA and RNA damage result in inhibited DNA synthesis and p53 activation.



CHEMOTHERAPY SIDE EFFECTS

Chemotherapy-induced gastrointestinal mucositis can result in a multitude of symptoms including ulceration, diarrhea, nausea, vomiting, bloating, and constipation. Mucositis symptoms are associated with 5-FU treatment. Severe mucositis can result in chemotherapy treatment reduction which presents a risk of decreased survival odds for the patient.

EFFECTS OF 5-FLUOROURACIL ON *BIFIDOBACTERIA*, *LACTOBACILLUS*, *BACTEROIDES*, AND *ESCHERICHIA COLI* IN VITRO

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ACKNOWLEDGEMENTS

Thank you to the Weber State Office of Undergraduate Research and the Mr. and Mrs. Denkers Family Foundation for funding this project. Thank you, also, to the Weber State Department of Medical Laboratory Sciences for use of the labs, Kent Criddle for ordering supplies and keeping track of orders, Kenton Cummins for letting us into the labs on weekends and holidays, and to Dr. Nicholaou for his support, advice, and expertise that have been invaluable to the project.

METHODS

Initially, organisms were tested with a range of 5-FU doses in ug/mL (1.56, 3.125, 6.25, 12.5, 25, 50, 100) included in which was an estimate of the average adult chemotherapeutic dose at the intestines (12.5 ug/mL). Organisms were grown in thioglycolate broth media with hemin at 0.005g/mL concentration in 15 mL tubes at the different 5-FU concentrations. SBA plates were streaked from the tubes after 24 hours and were incubated anaerobically for 24 hours. A colony count was performed for each plate. Growth inhibition was not observed in any of the test organisms.



RESULTS

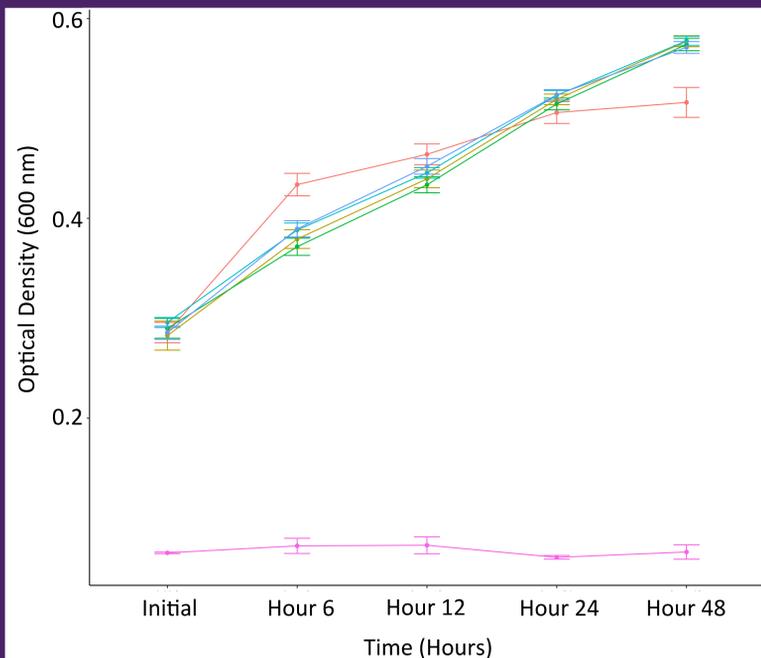


Figure 1. *Bifidobacteria* growth curve over 48 hours.

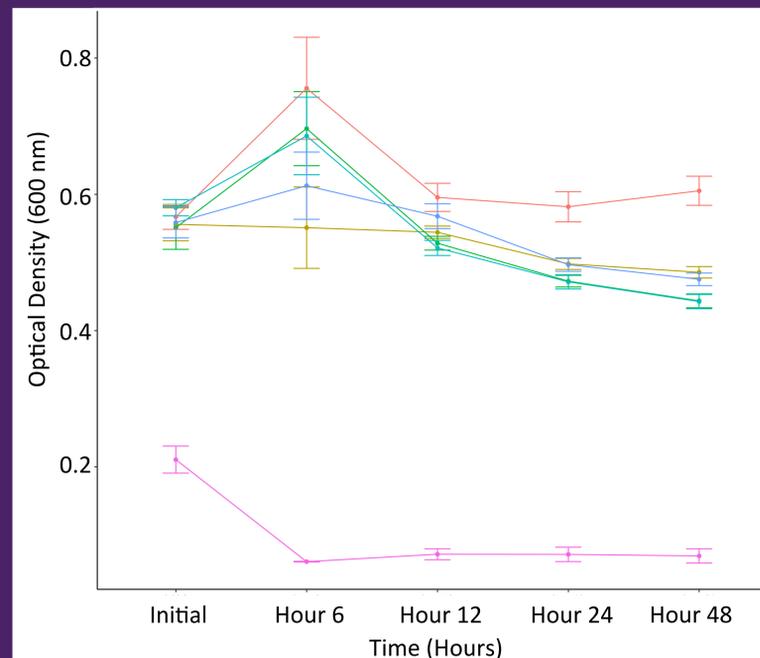


Figure 2. *Lactobacillus* growth curve over 48 hours.

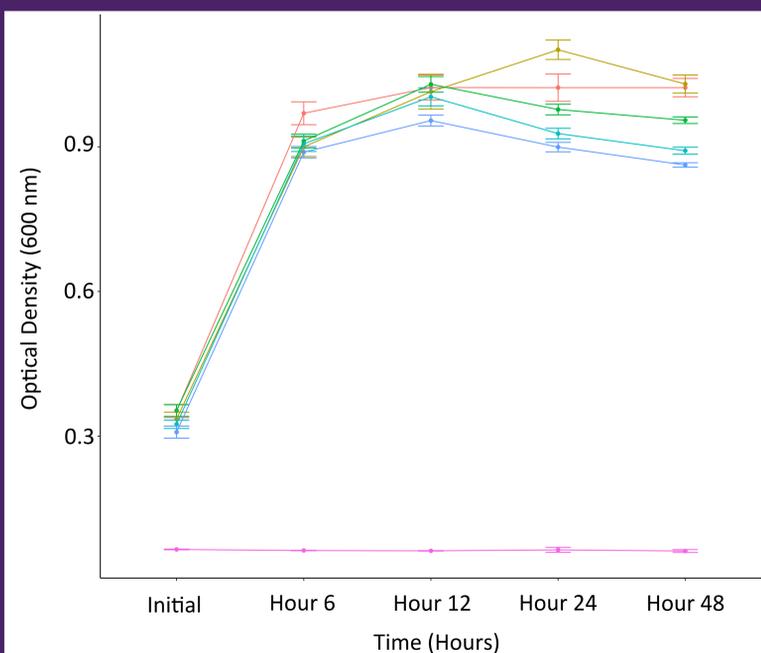


Figure 3. *Bacteroides* growth curve over 48 hours.

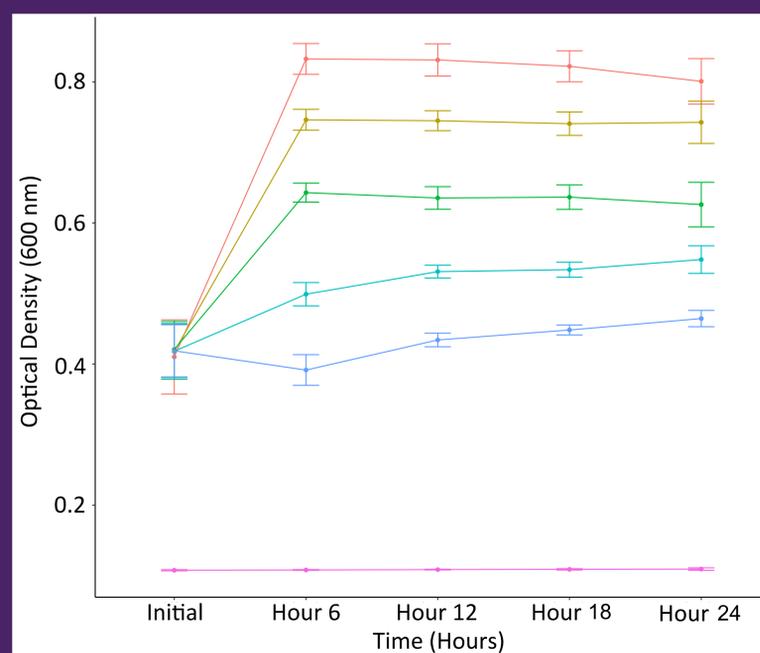


Figure 4. *Escherichia coli* growth curve over 24 hours

Figure Legend. Concentrations (ug/mL): growth: red, 75: gold, 150: green, 300: aqua, 540: blue, no growth: pink

DISCUSSION

In contrast to findings of previous *in vivo* human and animal studies, growth of *Escherichia coli* was inhibited by 5-FU proportionally to the dose of the drug. *Lactobacillus sp*. showed slight growth inhibition. Inhibition of growth was not seen in *Bifidobacteria sp* or *Bacteroides sp*. Differing cell wall structures may contribute to the varying effects of 5-FU on the normal intestinal organisms. Cell-signaling pathways unique to certain genera and species could contribute to different reactions as well. *In vivo*, cell-signaling between bacterial species and human cells is significant for cell function. Future studies could use cell cultures and stool cultures for *in vitro* testing to better mimic the cellular intestinal environment. Future studies will help determine other contributing factors to the decrease in intestinal organisms during 5-FU and other chemotherapeutic treatment in order to find a solution to quell adverse symptoms of chemotherapy-induced gastrointestinal mucositis.

5-FU was increased to doses that far exceeded those found in the human intestine following chemotherapeutic treatment. Tests were performed in optical 96 microwell plates using the same media. 5-FU was added by serial dilution at the following concentrations (ug/mL): 75, 150, 300, and 540. Organism was added to a .5 McFarland standard in each micro well for a total volume of 200 uL. For the strict anaerobes: *Bifidobacteria sp*, *Lactobacillus sp*, and *Bacteroides sp*., microwell plates were incubated anaerobically in anaerobic growth chambers using gas packs and methylene blue indicator strips. *Escherichia coli* was incubated aerobically in the Epoch 2 plate reader. "Growth" and "no growth" wells were used for controls and comparison. The Epoch 2 plate reader was used to read the optical density (OD) of each well at 600 nm to measure growth at the following time points: 0, 6, 12, 18*, 24, and 48** hours. *18 hour time point was used for *Escherichia coli* only. **48 hour time point was used for the slower growing strict anaerobes: *Bifidobacteria sp*, *Lactobacillus sp*, and *Bacteroides sp*.