Minimizing Chemotherapy Induced Intestinal Damage by Targeting Cell pH and Volume

Kendall Annetti, Nancy Walker, Ashlee Williams, Lane Clarke
Department of Biomedical Sciences, University of Missouri

**INTRODUCTION**

Chemotherapy induced intestinal damage occurs in 10-40% of patients.

Intestinal cell damage decreases quality of life and effectiveness of treatments.

Chemotherapy agents kill proliferating cells, inadvertently targeting intestinal stem cells (ISC) leading to inflammation.

ISC proliferation can be reversibly manipulated by altering intracellular pH and cell volume.

Enterooids form from isolated ISC that produce all four intestinal cell lineages, and generate crypt structures that are indicative of proliferating stem cells.

**OBJECTIVE**

Optimize the methods for growing and treating enteroids with doxorubicin for subsequent testing of hypothesis

**RESULTS**

- Isolated crypts from wild type mice were plated in stem cell medium with high concentrations of WrnCa (100 ng/mL) and R-spondin (500 ng/mL), which allows ISC to survive and proliferate, but not differentiate.
- After two or three days of stem cell medium, ISCs were treated with differentiation medium to allow enteroid formation.
- Live (budding) and dead (non-budding) enteroids were counted every day for 4 days.

**CONCLUSIONS**

Optimized methods for testing hypothesis that chemotherapy-induced intestinal damage can be minimized by manipulating ISC proliferation.

**HYPOTHESIS**

ISC acidification by facilitating HCO₃⁻ efflux or inhibiting H⁺ efflux will reduce proliferation during chemotherapy exposure and prevent ISC damage.

Subsequent alkalinization will enhance ISC proliferation during the recovery phase, minimizing intestinal damage.

**OPTIMIZE**

- Fluorescence of cells was measured at 485 emission/607 excitation after 1 hour to mimic peak serum levels of doxorubicin.

- Doxorubicin (0 to 3.0 µM) in differentiation medium was applied to immortalized human colon cancer cells (RKO cells).

- Fluorescence of cells was measured at 485 emission/607 excitation after 1 hour to mimic peak serum levels of doxorubicin.

- Doxorubicin could be measured down to a concentration of 0.1 µM.