Minimizing Chemotherapy Induced Intestinal Damage by Targeting Cell pH and Volume

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INTRODUCTION

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

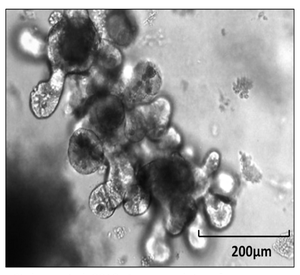
Intestinal cell damage decreases quality of life and effectiveness of treatments.

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.

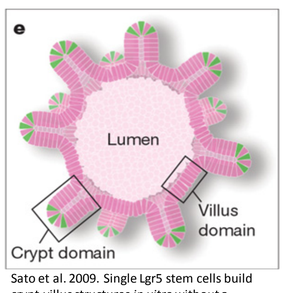
Enteroids form from isolated ISC that produce all four intestinal cell lineages, and generate crypt structures that are indicative of proliferating stem cells.

Cultured enteroid



Stelzner et al. 2012. A nomenclature for intestinal in vitro cultures. Gastrointestinal and Liver Physiology. 302:G1359-63.

Enteroid model



Sato et al. 2009. Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. Nature 459:262-266

HYPOTHESIS

ISC acidification by facilitating HCO_3^- 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.

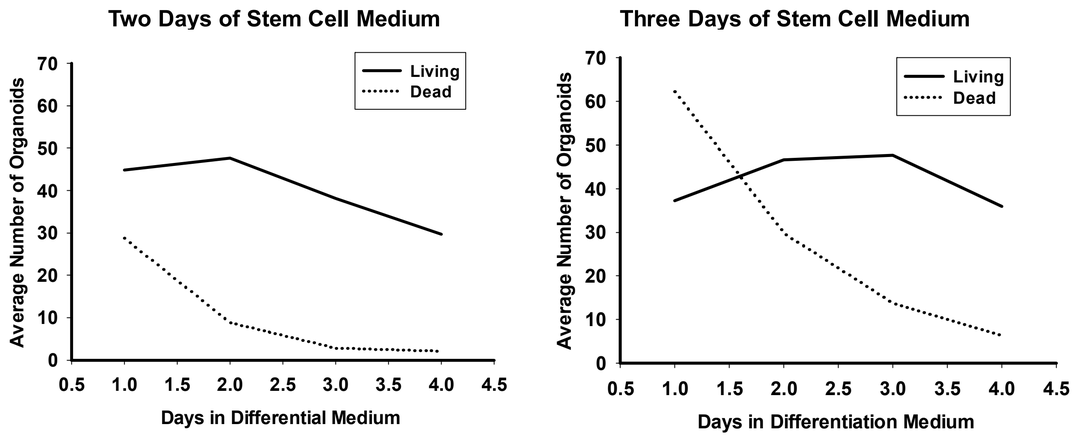
OBJECTIVE

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

RESULTS

Enteroid Survival Study

Isolate and culture ISC to get the greatest ratio of living to dead enteroids

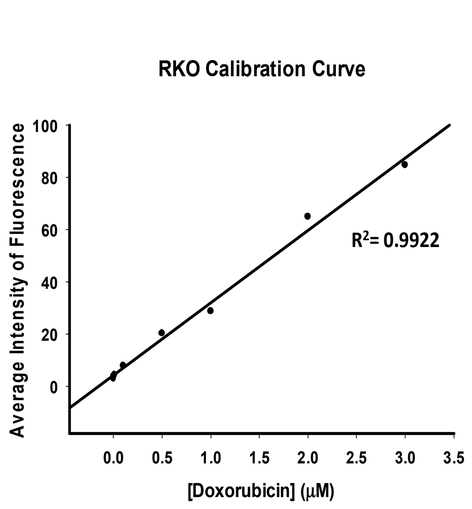


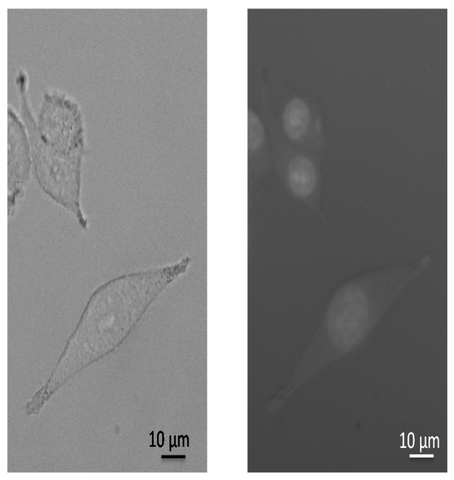
- Isolated crypts from wild type mice were plated in stem cell medium with high concentrations of Wnt3a (100 ng/mL) and R-spondin (500 ng/mL), which allows ISC to survive and proliferate, but not differentiate
- After two or three days of stem cell medium, ISCs were treated with differentiation medium to allow enteroid formation
- Live (budding) and dead (non-budding) enteroids were counted every day for 4 days

Incubating for 3 days in stem cell medium and 2 days in differentiation medium results in the best live to dead ratio

Calibration of Doxorubicin Bioassay

Measure residual doxorubicin in enteroids after treatment





Light and fluorescence images of RKO cells with 1.0 µM doxorubicin (40x objective, 10000 ms exposure)

- Doxorubicin (0 to 3.0 uM) 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

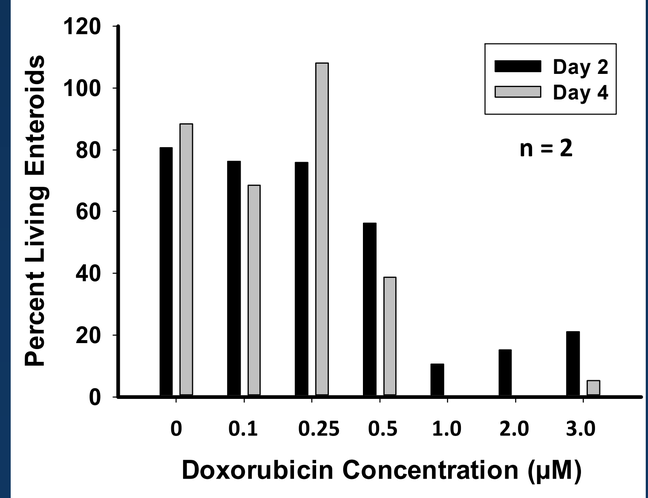
Doxorubicin could be measured down to a concentration of 0.1 µM

Doxorubicin Dose Response

Determine the LD_{50} concentration of doxorubicin in enteroids

- ISC were isolated and plated in differentiation medium for 3 days pre-treatment
- Doxorubicin (0 to 3.0 uM) was added to the medium for 1 hour, then washed off
- Live and dead enteroids were counted on day 0 (pre-treatment), and day 2 and day 4 (post-treatment)
- Percent surviving enteroids was calculated by diving living enteroids on each day by the number of enteroids pre-treatment

Doxorubicin Dose Response



The LD_{50} of doxorubicin on enteroids is approximately 0.5 µM

CONCLUSIONS

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

Created methods to reproducibly culture enteroids, measure residual doxorubicin post-treatment, and treat enteroids with an LD_{50} concentration of doxorubicin.

Future Studies: Manipulate intracellular pH and volume to prevent chemotherapy induced intestinal damage

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