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BSc Honours (Biology), Dalhousie University, 2011
MSc (Pharmacology), Dalhousie University, 2013

DEPARTMENT OF MICROBIOLOGY & IMMUNOLOGY

TITLE OF THESIS:	Developing a Two-pronged Drug Screen to Identify Compounds that Protect against Cisplatin-induced Oto- and Nephrotoxicity
TIME/DATE:	9:30 am, Wednesday, November 13, 2019
PLACE:	Room 3107, The Mona Campbell Building, 1459 Lemarchant Street

EXAMINING COMMITTEE:

Dr. David Raible, Department of Biology, University of Washington (External Examiner)

Dr. Graham Dellaire, Department of Pathology, Dalhousie University (Reader)

Dr. Sidney Croul, Department of Pathology, Dalhousie University (Reader)

Dr. Meredith Irwin, SickKids Hospital (Reader)

Dr. Jason Berman, Department of Pediatrics, University of Ottawa (Co-Supervisor)

Dr. Craig McCormick, Department of Microbiology & Immunology, Dalhousie University (Co-Supervisor)

DEPARTMENTAL REPRESENTATIVE:	Dr. Roy Duncan, Department of Microbiology & Immunology, Dalhousie University
CHAIR:	TBD, PhD Defence Panel, Faculty of Graduate Studies

ABSTRACT

Cisplatin is a chemotherapy used to treat a variety of cancers, including several pediatric malignancies. Cancer survivors are plagued by lifelong cisplatin-induced toxicities, including kidney damage and ototoxicity, or hearing loss. In efforts to find adjuvant compounds that can prevent these toxicities, in this study, I employed a two-pronged oto- and nephrotoxicity drug screen. The zebrafish is an excellent model for studying drug toxicities due to conserved genetics and organ systems. Zebrafish have lateral line neuromasts, analogous to clusters of mammalian cochlear hair cells. Similar to hair cell death in humans, cisplatin exposure causes decreases in neuromast viability, visualized with YO-PRO1, a fluorescent dye. Larvae treated with cisplatin (0.001-0.05 mM) demonstrated a dose-dependent reduction in neuromast fluorescence that I was able to measure rapidly using a biosorter. Zebrafish larvae also possess pronephros structures similar to the mammalian nephron. Glomerular filtration rate (GFR) can be measured in larvae by injecting FITC-tagged inulin into circulation. Inulin is renally-excreted, so decreases in vascular fluorescence approximates GFR. Cisplatin-treated larvae exhibited a reduction in GFR, which was also detectable with the biosorter. I employed an *in vivo* larval neuromast screen to assess the effects of compounds from the Sigma LOPAC®1280 library on cisplatin-induced ototoxicity. I compared these results with an *in vitro* toxicity test of cisplatin +/- the compound library on human proximal tubule cells, and found 22 drugs that were effective in both assays. I validated the protective capacity of two of these adjuvants, dopamine and L-mimosine, with confocal microscopy of the inner ear and the GFR assay. To determine if these protective compounds impact cisplatin's cytotoxic effects, I performed an alamarBlue™ viability assay, an apoptosis-based flow cytometry assay, and a γ-H2AX-based immunohistochemistry assay designed to detect double strand breaks (DBSs) in DNA. In each of these assays, dopamine and L-mimosine did not reduce the cytotoxicity of cisplatin in SK-N-AS and LAN5 neuroblastoma (NBL) cells, or HSC-3 oral squamous cell carcinoma cells. This project provides technological advances in the field of zebrafish-based drug screening, and identifies dopamine and L-mimosine as candidate oto- and nephroprotective compounds.