DNA damage response pathways are induced after exposure to various heavy metals in *C. elegans*

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Abstract

A major route of exposure to various heavy metals is through contaminated soil and water. Research has shown that these substances play roles in the induction of various diseases such as cancer, neurodegeneration and birth defects. In the cell, proteins such as metallothioneins respond to heavy metal exposure and chelate the metal to prevent cellular damage. However, little is known about the cellular response in regards to DNA damage after heavy metal exposure. To provide a better understanding of this cellular response, the induction of both cell cycle arrest and apoptosis were investigated after exposure to copper, cadmium, iron, lead, nickel and silver in the nematode *C. elegans*. Growth assays were conducted to determine EC10 and EC50 concentrations, which were utilized to investigate if DNA damage response pathway(s), apoptosis and/or cell cycle arrest, were being induced upon exposure. For all metals, apoptosis was induced in the germline with copper, nickel and iron resulting in the greatest induction. To determine if the induction of apoptosis is p53 dependent and thus due to DNA damage, a *cep-1* mutant is being tested. Preliminary data shows that lead and cadmium but not silver, copper, and iron induce p53 dependent apoptosis suggesting DNA damage is occurring in the cell. Cell cycle arrest was also induced in the germline for all metals. Nickel, silver and iron resulted in the most arrested cells. Apoptosis and cell cycle assays for all metals tested will allow us to better understand the damage being caused by the metal exposure as well as mechanisms induced by the cell in response to exposure.

Background

Exposure to Heavy Metals

- Found in environment and introduced through mining and disposal
- Major routes of exposure through contaminated water and food (grains, leafy greens and seafood are most common)

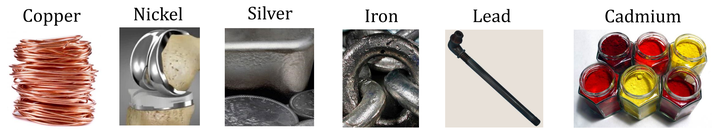
Heavy Metal Toxicity

- Very effective at generating oxidative stress and producing reactive oxygen species (ROS)
- Causes damage to biomolecules, subcellular compartments and even whole cells
- At cytotoxic levels are known to be teratogenic, cause neurodegenerative diseases and cancer and some have been linked to aging

DNA damage response pathways

- ROS can damage DNA and excessive DNA damage leads to the induction of pathways that induce cell cycle arrest or apoptosis
- Cell cycle arrest allows for the repair of damage
- Apoptosis or programmed cell death can be p53 dependent or independent

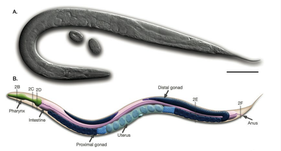
Heavy Metals being investigated



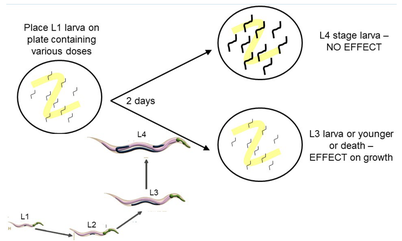
Methods

C. elegans as a model organism

- Non-parasitic nematode
- ~ 1 mm in length
- Hermaphrodites provide homogenous population
  - Rare males allow for genetic crosses
- 3.5 day developmental cycle
- ~300 progeny per hermaphrodite
- Cell and developmental biology are understood in exceptional detail
- DNA damage studies established
- Toxicological assays established
- Sequenced genome and orthologous gene expression



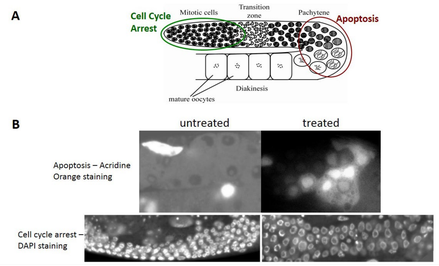
Growth analysis to determine EC10 and EC50



Growth in response to various concentrations was measured after 48 h exposure. A curve was created based on responses. The EC10 and EC50 for each metal was determined based on the growth curve.

Metal used for experiments include: Copper (III) chloride; Nickel sulfate; Silver nitrate; Lead nitrate; Iron nitrate; and Cadmium chloride

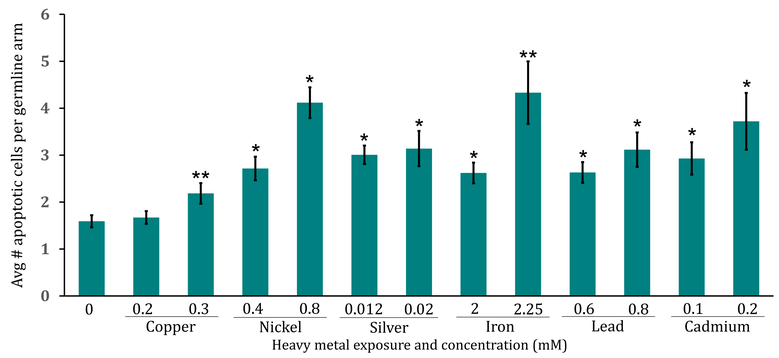
Apoptosis and Cell cycle arrest assays to investigate the induction of DNA damage response pathways



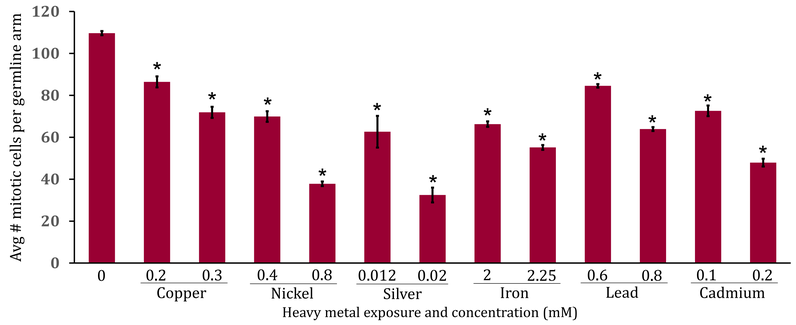
(A) Cell cycle arrest was observed in the distal tip of the germline and apoptosis was measured in the bend. (B) L4 nematodes were exposed to heavy metal for 24h and subsequently stained with acridine orange for apoptosis (upper) or DAPI for cell cycle arrest (lower). Presented are apoptosis in response to 120Gy IR and cell cycle after exposure to hydroxyurea.

Results

Apoptosis is induced in a dose dependent manner

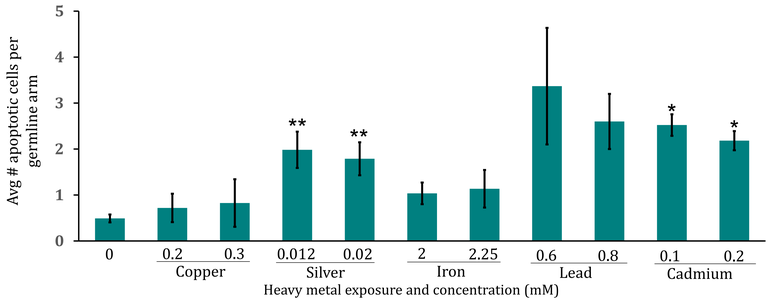


Cell cycle arrest is induced in a dose dependent manner



* p<.01 compared to untreated as determined by a two-tailed t-test
Data presented is avg. +/- S.E.M. for n = 4-7 biological replicates

Investigating if apoptosis induction is p53 dependent using *cep-1*



cep-1 is a p53 mutant strain
* p<.01 and ** p<.05 compared to untreated as determined by a two-tailed t-test
Data presented is avg. +/- S.E.M. for n = 5-12 biological

Apoptosis induction in response to copper, silver, and iron is p53 dependent

Heavy metal exposure and concentration (mM)		Avg # apoptotic cells per germline arm		*p*-value N2 compared to *cep-1*	p53 dependent apoptosis
		N2	*cep-1*		
Copper	0.2	2.19	0.72	0.05	Yes
	0.3	2.19	0.79	0.04	
Silver	0.012	3.01	1.98	0.06	Yes
	0.02	3.14	1.79	0.03	
Iron	2	2.62	1.04	0.01	Yes
	2.25	4.33	1.14	0.01	
Lead	0.6	2.63	3.37	0.66	No
	0.8	3.12	2.6	0.54	
Cadmium	0.1	2.93	2.52	0.35	No
	0.2	3.72	2.18	0.04	

Discussion

- ✓ All heavy metals, except 200 µM of copper, resulted in a significant increase in apoptosis after exposure
- ✓ All heavy metals resulted in a significant decrease in the number of mitotic cells in the germline after exposure suggesting the induction of cell cycle arrest
- ✓ In some cases, specifically copper and silver, the determined EC50 resulted in unhealthy and developmentally delayed L4 hermaphrodites. A concentration at approximately the EC20 was subsequently used for the apoptosis and cell cycle assay.
- ✓ Nickel and copper exposed nematodes displayed abnormal germline morphology, specifically in regards to the embryos
  - Reproduction assay concluded that both nickel and copper resulted in a significant decrease in brood size and an increase in embryonic lethality
- ✓ Lead and cadmium induce apoptosis in the absence of p53 at a level not significantly different than wildtype suggesting that the induction of apoptosis is p53 independent

Future Directions

- ✓ Finish, for all metals, if the mechanism of apoptosis induction is p53 dependent or independent
- ✓ Investigate pathways of cell cycle arrest
- ✓ Investigate reproduction effects after exposure

Acknowledgements

This research was funded by the Lincoln Memorial University Mini-Grant, the Department of Biology and the Master of Science program with the School of Math and Science at Lincoln Memorial University.

Project was initiated as part of Senior Honors thesis projects by undergraduate students Kaitlin Campbell (silver and nickel) and Megan McMurray (iron and lead) at LMU. Continuation and data for apoptosis and cell cycle assays was obtained by various Master of Science students.