

Organophosphate Exposure Alters Protein Expression by Equine Adult Multipotent Stromal Cells

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INTRODUCTION: Chlorpyrifos (CPF) is an acetylcholinesterase inhibitor, an insecticide class frequently associated with toxicosis. Proteomic analysis was performed after short term exposure of equine adipose tissue-derived multipotent stromal cells (ASCs) to CPF to assess potential impacts on musculoskeletal maintenance and healing.

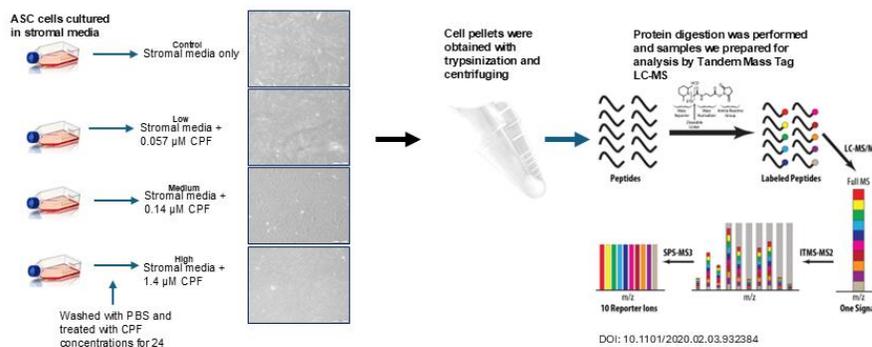
METHODS: Passage 2 ASCs were exposed to 0, 0.057, 0.14, or 1.4 μM CPF in stromal media for 24 hours (Fig. 1). Tandem mass tag labeling mass spectrometry-liquid chromatography was used to quantify protein levels. Protein identifications were accepted with less than 1.0% false discovery and at least 2 identified peptides. Significant changes in individual protein expression were quantified ($p \leq 0.05$). Protein up- and down-regulation relative to control (no exposure) were determined for each CFP concentration and compared among concentration cohorts.

RESULTS: Relative to control, 97 proteins were up- (34) or down- (63) regulated. Five proteins were altered at all three concentrations; three (ZEB2, ABI2, BRCC3) were upregulated and two (ADARB1, CFAP20) were downregulated. Ephrin B1 was upregulated at 0.057 μM and downregulated at 0.14 μM and 1.4 μM concentrations vs control. It was upregulated at 0.057 μM vs 0.14 μM and 1.4 μM CPF. Affected proteins were primarily involved in cell motility, metabolism, and nervous system maintenance.

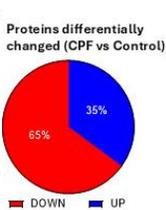
DISCUSSION: Findings provide information on the impact of CPF exposure on native stem cells. Results are limited to the CFP concentrations and single time point evaluated.

SIGNIFICANCE/CLINICAL RELEVANCE: Altered ASC protein expression from CPF exposure could inhibit the ability to mature into cell phenotypes for homeostasis and repair of musculoskeletal innervation

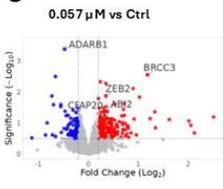
A



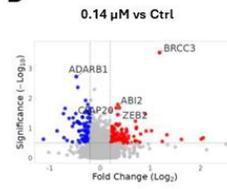
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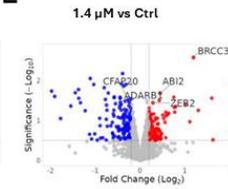
C



D



E



F

Gene ID	Upregulated at all CPF concentrations vs Ctrl		Downregulated at all CPF concentrations vs Ctrl	
	P. value	Gene ID	P. value	
ZEB2	0.014	ADARB1	0.000	
	0.036		0.002	
	0.036		0.035	
ABI2	0.043	CFAP20	0.046	
	0.021		0.045	
	0.021		0.030	
BRCC3	0.003			
	0.000			
	0.003			

A. Schematic of equine-ASC culture, chlorpyrifos exposure, and protein isolation for LC-MS analysis. B. Pie chart of protein percentages upregulated or downregulated ($p \leq 0.05$). C-E: Volcano plot of all proteins with differential protein expression after 24 hours of chlorpyrifos exposure at concentrations of 0.057 μM (C), 0.14 μM (D), or 1.4 μM (E). Blue points represent significantly downregulated proteins. Red points represent significantly upregulated proteins ($p < 0.05$). F. Table of up- and down-regulated genes across all treatment groups that were upregulated and downregulated. ZEB2=Zinc Finger E-Box Binding Homeobox 2; ABI2=Abl Interactor 2; BRCC3=BRCA1/BRCA2-Containing Complex Subunit 3; ADARB1=Adenosine Deaminase RNA Specific B1; CFAP20=Cilia And Flagella Associated Protein 20.