Sanford Burnham Prebys Metabolomics Core: Data Report [SECIM SUBMISSION]

Date: 06/08/2016	**SBMRI Project #**: 16-010	**PI:** David Wald

Biological Samples Submitted

Species: Human
Sample Type: HL-60 Cells (Leukemia Cells)

Experimental Variables: Control, N = 5
Malate dehydrogenase (MDH) inhibited, N=5

N: 5 per group (Total N = 10)
Cells were treated for 15 hr with a MDH inhibitor or vehicle.

Sample Preparation

Frozen cells were received on dry ice and stored at -80° C. Before the assay was performed, the samples were lyophilized to dryness. The lyophilized pellet was homogenized in 150 µL of 50/50 (acetonitrile/0.3% formic acid) using a Precellys (bead beating) system. A 50 µL aliquot of the homogenate was used to performed the organic acids assay.

Assay Modules Performed

Organic Acids

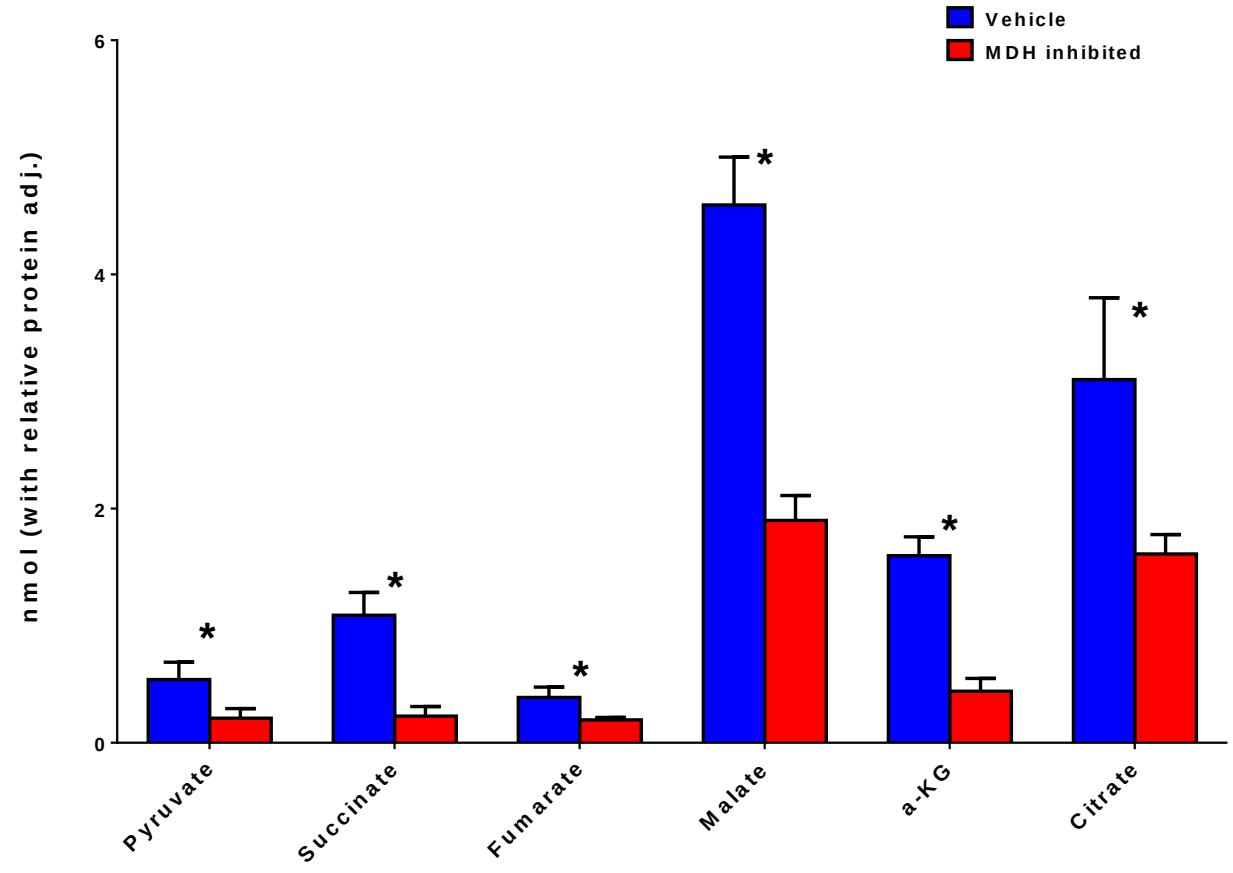
Sample Preparation and LC-MS: AZ
Data Analysis and Reporting: AZ and SJG
Instrumentation: Thermo Quantiva LC/MS/MS

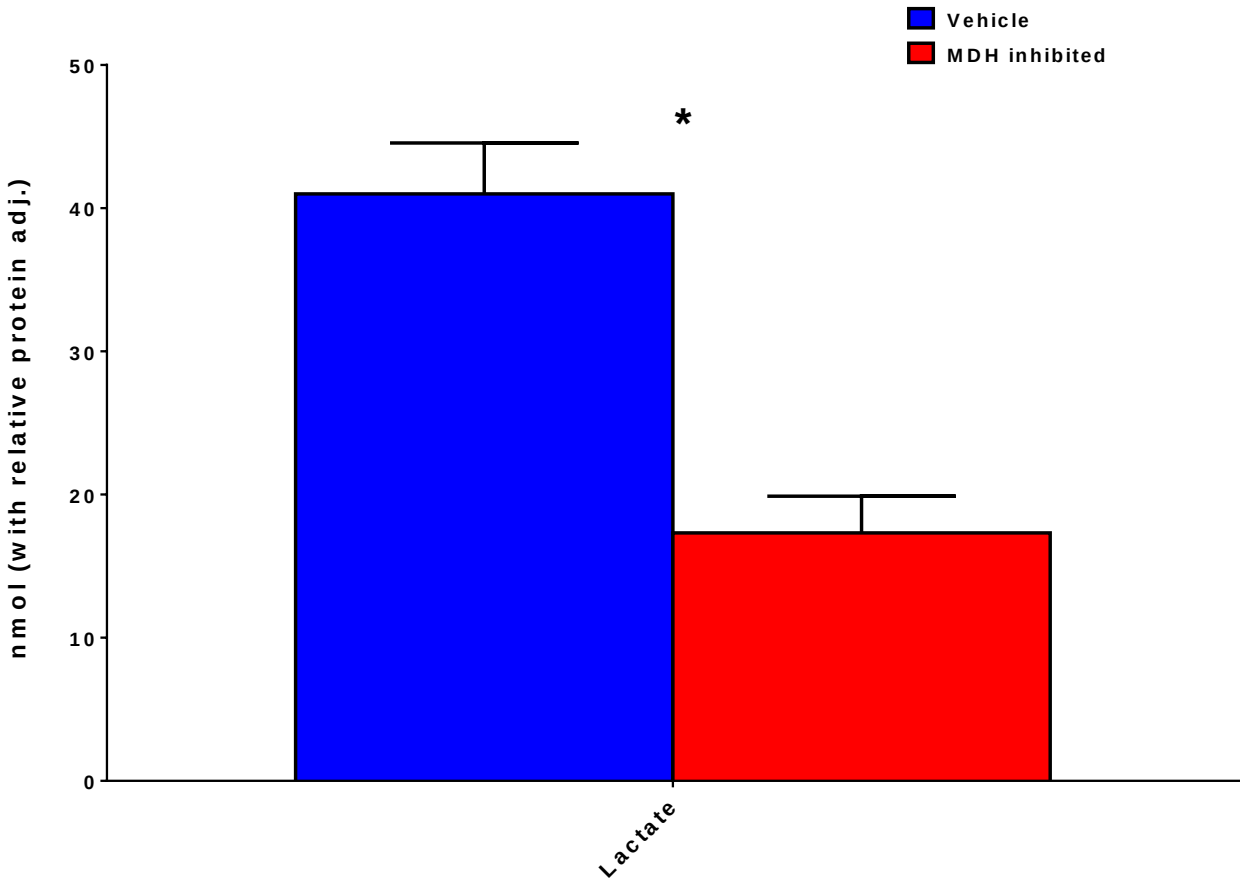
Used authentic heavy isotope-labeled internal standards for all organic acids.

Background Information

Malate dehydrogenase is an enzyme that reversibly catalyzes the oxidation of malate to produce oxaloacetate using the reduction of NAD^+ to NADH. There are two main isoforms in eukaryotic cells. One is found in the mitochondrial matrix, participating as a key enzyme in the TCA cycle that catalyzes the oxidation of malate. The other is found in the cytoplasm, assisting the malate-aspartate shuttle with exchanging reducing equivalents so that malate can pass through the mitochondrial membrane to be transformed into oxaloacetate for further cellular processes

Nucleotides
Vehicle vs. MDH inhibited
N = 5, 5
Note: * designates significant differences ($p < 0.05$)





SIGNIFICANT CHANGES

The following tables depict the analytes that display statistically significant changes. All P values were determined by a Student's t-test.

	MDH inhibited vs. Vehicle	
	% change	P value
Lactate	-57.8	< 0.001
Pyruvate	-60.7	0.002
Succinate	-78.9	< 0.001
Fumarate	-49.1	0.001
Malate	-58.6	< 0.001
a-KG	-72.5	< 0.001
Citrate	-48.0	0.012

COMMENTS

Highly significant and marked reductions of the organic acids were observed in cells that were treated with the malate dehydrogenase inhibitor.

Note: we do not measure oxaloacetic acid (OAA) which would be very worthwhile for this investigation since it is a substrate/product for malate dehydrogenase. OAA is known to be labile – which makes it crucial to use a heavy isotope labeled internal standard which is not yet commercially available. We have alerted one of our commercial vendors and, hopefully, the OAA internal standard can be available in the not too distant future.

We did have two questions:

Are both isoforms inhibited by the malate dehydrogenase inhibitor?

Was there any evidence for cell toxicity in the presence of treated with the malate dehydrogenase inhibitor?