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Neurotoxicity Screening of Antiretroviral Drugs With Human iPSC-Derived Neurons

Sandy Hinckley[1], Sean Sherman[1], Brookie Best[2], Jeremiah Momper[2], Qing Ma[3], Scott Letendre[2], Ronald Ellis[2], & Anne Bang[1]

[1]Sanford Burnham Prebys Medical Discovery Institute, [2]University of California San Diego, [3]State University of New York at Buffalo

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UC San Diego School of Medicine

Introduction

While antiretroviral therapy (ART) has become increasingly effective and well-tolerated, there remains a patient subset that experience central nervous system (CNS) side effects, and neurocognitive performance may actually improve following change in specific ART agents. Understanding the risk associated with these drugs will allow informed selection of optimal therapy. Using human induced pluripotent stem cell-derived neurons (hiPSC), we screened 10 ART drugs and generated a neurotoxicity profile based on mitochondrial membrane potential, reactive oxygen species, cell health and neurite growth. The ART drugs in our screening set were chosen for their characterization as the current recommended (ABC, TDF, DRV, DTG, EVG) or first alternative (EFV, RPV, ATV) treatment for antiretroviral-naïve adults[1].

1 Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. Available at http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. Accessed February 21, 2016. Page 58, Table F3

Methods

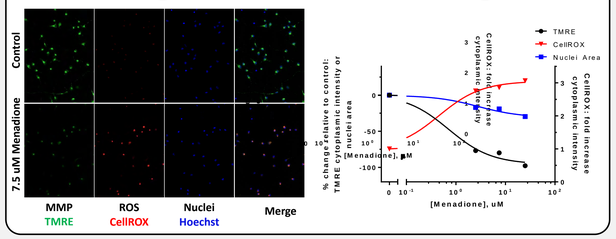
10 antiretroviral therapy (ART) drugs were acquired by the NIH AIDS drug repository. hIPSC derived iCell cortical neurons (Cellular Dynamics International) were plated on high throughput 384 well plates for assay screening. Both neurite growth and mitochondrial function were assessed on the Opera High Content Screening System (Perkin Elmer) and analyzed with Acapella software. The 10 ARV drugs in addition to the control (Menadione) were screened for mitochondrial function in a multiplexed, live cell assay. iCell neurons were loaded with the mitochondrial membrane potentiometric dye TMRE and reactive oxygen species sensor CellROX deep red. The cells were exposed to drugs in 7-point dose response in duplicate for 4 hours followed by Hoechst stain and imaging. The 10 ARV drugs in addition to the controls (Staurosporine and BIO) were screened for neurite growth after a 3 day drug exposure in 7-point dose response (n=4 wells/dose). Neurons were fixed and immunostained for the neuronal marker TUJ1 and nuclei marker Hoechst. Statistical significance was determined as Z-score greater than 2 standard deviations from the mean of the DMSO control.

Antiretroviral Screening Set



		Assay		Plasma	
	Drug	Mitochondrial	Neurite	Average steady state []	Protein binding
NRTI	Abacavir	0.15-150uM	0.033-33uM	1.75uM	50%
	Tenofovir	0.25-25uM	0.033-10uM	0.91uM	0.7-7.2%
NNRTI	Efavirenz	0.15-150uM	0.033-33uM	7.6uM	99.5-99.75%
	Rilpivirine	0.10-50uM	0.033-33uM	0.25uM	99.7%
INSTI	Elvitegravir	0.15-150uM	0.033-33uM	1.67uM	98-99%
	Dolutegravir	0.075-75uM	0.033-33uM	7.44uM	99%
PI	Atazanavir	0.15-150uM	0.033-33uM	2.96uM	86%
	Darunavir	0.15-150uM	0.033-33uM	7.68uM	95%
PK enhancer	Ritonavir	0.15-150uM	0.033-33uM	7.88uM	98-99%
	Cobicistat	0.15-150uM	0.033-33uM	0.39uM	97-98%

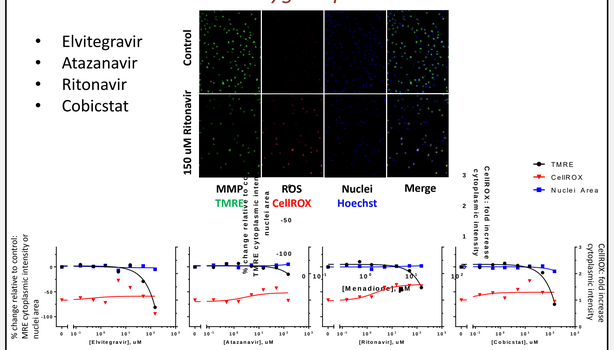
Mitochondrial Toxicity High Content Screening



Mitochondrial Toxicity Of Antiretroviral Drugs

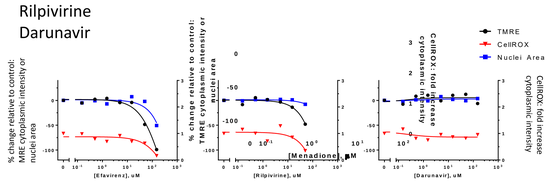
Mitochondrial Membrane Potential Depolarized and Reactive Oxygen Species Generated

- Elvitegravir
- Atazanavir
- Ritonavir
- Cobicistat



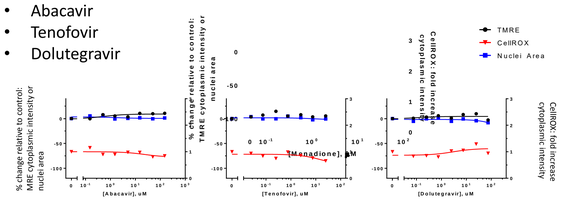
Mitochondrial Membrane Potential Dysfunction

- Efavirenz
- Rilpivirine
- Darunavir

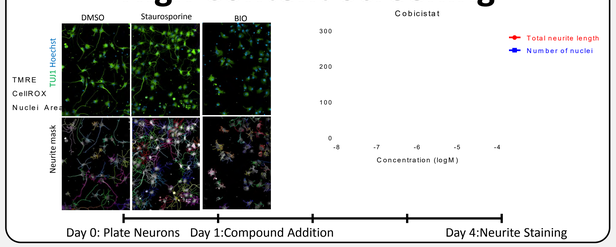


No Effect on Mitochondrial Function or Cell Health

- Abacavir
- Tenofovir
- Dolutegravir



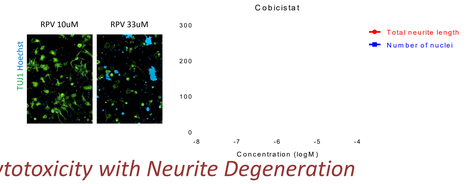
Neurite Outgrowth High Content Screening



Neurite Outgrowth During Antiretroviral Drug Treatment

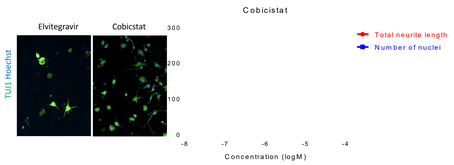
Neurite Retraction

- Rilpivirine



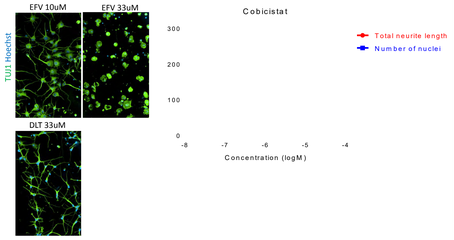
Cytotoxicity with Neurite Degeneration

- Elvitegravir
- Cobicistat



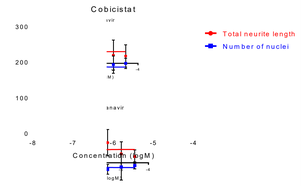
Neurite Outgrowth

- Efavirenz
- Dolutegravir

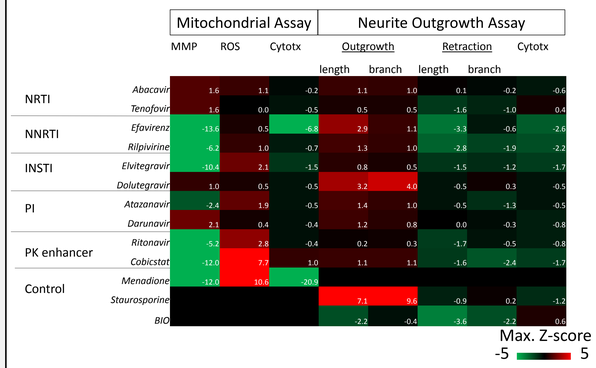


No Effect on Neurite Growth or Cell Health

- Abacavir
- Tenofovir
- Atazanavir
- Darunavir
- Ritonavir



Results Overview



- NNRTI and EVG were mitotoxic resulting in neurite retraction/cytotoxicity
- In contrast, PI were mitotoxic but did not significantly impact neuronal morphology or long-term cell health

	Min effective dose		
	Drug	Mito	Neurite
NRTI	Abacavir	0	0
	Tenofovir	0	0
NNRTI	Efavirenz	50uM	10uM
	Rilpivirine	33uM	10uM
INSTI	Elvitegravir	5uM	33uM
	Dolutegravir	0	33uM
PI	Ritonavir	15uM	0
	Atazanavir	150uM	0
	Darunavir	50uM	0
PK enhancer	Cobicistat	5uM	33uM

Conclusions

We characterized toxicity of 10 ART drugs and linked the majority with impacts either on mitochondrial function, neurite growth, or both. Although immature, hiPSC neurons are human and scalable for drug screening. Further studies are needed to determine whether our *in vitro* assays reflect neurotoxicity *in vivo,* but our results suggest that increased drug concentrations in the CNS could have adverse clinical consequences.

- NNRTIs were strongest neurotoxins in drug set
- 8/10 antiretroviral drugs had a direct effect on neuronal mitochondrial function, morphology, and/or health

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