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Integrating Cytotoxicity Profiling into the H295R Steroidogenesis Assay

A.L. Karmaus[1], N. Christy[1], J. Fowler[1], and S. Levine[2]

[1]ILS, Morrisville, NC, USA; [2]Bayer U.S. Crop Science, Chesterfield, MO, USA

Agnes Karmaus
akarmaus@ils-inc.com

Highlights

To help understand mechanisms of chemical-mediated disruption in steroid hormone biosynthesis (steroidogenesis), a panel of cytotoxicity assays was used. Assays probing cell membrane integrity, redox enzyme capacity, and mitochondrial status with varying sensitivities were used.

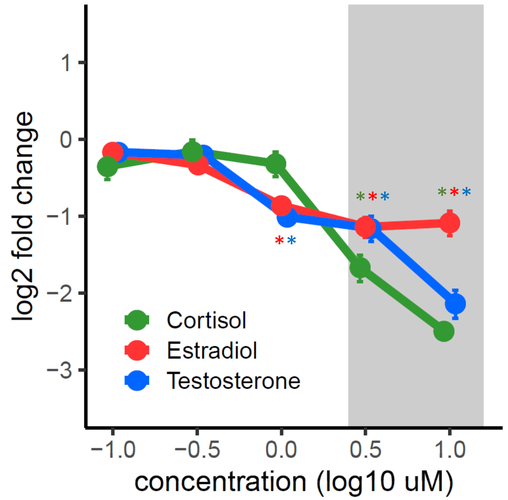
CCCP, a mitochondrial uncoupling agent, was used in this proof of concept:

- CCCP decreased hormone levels for cortisol, testosterone, and estradiol.
- The panel of cytotoxicity assays revealed that JC-10 was most sensitive in detecting mitochondrial disruption (loss of membrane potential), more so than MTT which relies on mitochondrial oxidoreductase enzyme activity).
- Assays probing cell membrane integrity or redox capacity did not detect diminished cell viability despite loss of mitochondrial membrane potential.

Altogether, comprehensive assessment of cytotoxicity, by probing cell and mitochondrial status, can complement hormone data to characterize mechanism of action or non-specific effects for chemical-mediated disruption of steroidogenesis.

Table 1: Cytotoxicity Assays and Mechanisms Evaluated

Cytotoxicity Assay	Mechanism
LDH-Glo	Cell membrane integrity (LDH leakage)
CellTiter-Blue	Redox reaction (mitochondrial, cytosolic, or microsomal enzymes)
CellTiter-Glo	Mitochondrial activity (ATP quantification)
MTT	Mitochondrial activity (NAD(P)H-dependent oxidoreductase enzyme activity)
JC-10	Mitochondrial membrane potential (dye)
MitoTracker	Mitochondrial membrane potential (dye)



Effects on Hormone Levels

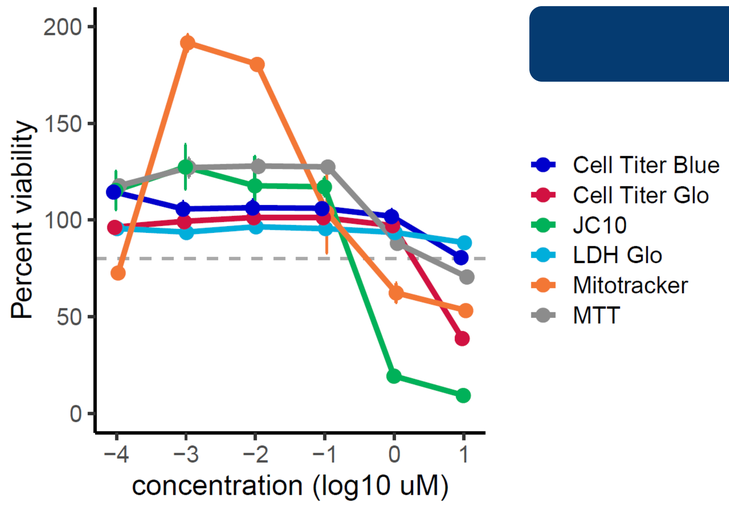
At the top concentration tested (10 µM), CCCP resulted in:

- Cortisol levels decreasing as low as 5.6-fold
- Testosterone levels decreasing as low as 2.1-fold
- Estradiol levels decreasing as low as 4.4-fold

(*) represent $p<0.05$ relative to plate-matched DMSO vehicle control by ANOVA with Dunnett's *post hoc* test (colors match hormones)

Cytotoxicity, assessed by MTT, was performed on the same cells from which hormone levels were evaluated

- At the top two testing concentrations (3 and 10 µM), CCCP resulted in 65% and 36% cell viability, respectively (shaded grey)



Cytotoxicity Profile

CCCP did not result in significant effects on cell viability as detected by CellTiter-Blue and LDH-Glo suggesting cells are intact and still have redox capacity at the time point and testing concentrations used

However, CCCP elicited significant effects in mitochondrial cell viability assays:

- MTT (70% cell viability; down 50% from baseline)
- MitoTracker (53% cell viability)
- CellTiter-Glo (40% cell viability)
- JC-10 was the most sensitive (10% cell viability)