Impact of Profiling Cytotoxicity in the H295R Steroidogenesis Assay

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INTRODUCTION

OECD Test Guideline 456 is a validated H295R steroidogenesis assay which utilises human adrenocortical carcinoma cells to evaluate chemical effects on testosterone (T) and 17β-estradiol (E2) production. This assay requires a parallel measure of cytotoxicity, generally by MTT assay. Given that the rate limiting and commitment steps for steroidogenesis depend on mitochondrial enzymes, it is informative to assess the state of the mitochondria to help inform on mechanism of action for potential steroidogenesis disruption; however, mitochondrial function and cell viability may not always be equal and redundant in this system (e.g. in senescent cells).

METHODOLOGY

We integrated a battery of cytotoxicity assays to inform on cell membrane integrity, mitochondrial function, and ATP quantification to comprehensively characterise chemical-elicited effects in H295R cell status. Using a custom set of ten reference chemicals, our evaluation included seven complementary assays to inform on potential modes of action for cytotoxicity. For example, we can assess which chemicals cause effects on cell membrane integrity (LDH-Glo) as opposed to mitochondrial effects such as depolarisation (JC-10; MitoTracker) or mitochondrial activity (CellTiter-Glo for ATP quantification and MTT to assess NAD(p)H-dependent oxidoreductase enzyme activity). Chemicals were evaluated at five test concentrations; after 48 hours of exposure, concentration-response effects on cell viability were assayed and T and E2 levels quantified.

RESULTS

As an example of the results, carbonyl cyanide m-chlorophenylhydrazone (CCCP), a mitochondrial uncoupling agent, resulted in decreased T and E2 levels and elicited significant effects in the CellTiter-Glo, MitoTracker, and most notably the JC-10 assay which detects mitochondrial membrane potential. These results confirm that CCCP impaired mitochondrial stability in H295R cells but did not cause overt cell death (with no significant effects detected by MTT, LDH Glo, or CellTiter Blue assays) within the timeframe of the

study. Thus, for CCCP, mitochondrial disruption likely underlies the observed decrease in hormone production.

DISCUSSION

Cumulatively, this study reveals that comprehensive assessment of cytotoxicity, as determined by probing cell and mitochondrial status, can complement hormone data to better understand and characterise the mechanism of action for chemical-mediated disruption of steroidogenesis.