Screening Chemical Effects on Steroidogenesis in H295R Human Adrenocortical Carcinoma Cells

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Abstract

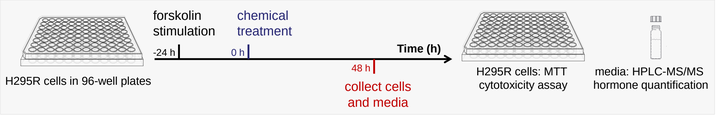
Proper endocrine function requires steroid hormone biosynthesis and metabolism (steroidogenesis). Disruption of steroidogenesis by environmental chemicals can result in altered hormone levels causing adverse reproductive and developmental effects. This study is the first to establish a high-throughput model to evaluate a diverse library of chemicals for effects on 13 major hormones in the steriodogenic pathway. Using H295R human adrenocortical carcinoma cells in a 96-well format, steroidogenesis was induced by pre-stimulation with 10 µM forskolin for 48 hr followed by chemical exposure for 48 hr. Media were removed and hormones were quantified by HPLC-MS/MS including progestagens (PROG, OHPROG, OHPREG), glucocorticoids (DOC, 11DCORT, CORTISOL), androgens (ANDR, TESTO), and estrogens (ESTRONE, ESTRADIOL). Initially, 2075 unique ToxCast chemicals were tested at a single non-cytotoxic concentration, of which 1112 chemicals (54%) altered levels of at least one measured hormone. Based on the single concentration analysis, 428 chemicals altering the levels of ≥4 hormones were selected for six-point concentration-response (0.003 – 100 µM). The concentration-dependent evaluation of 13 hormones not only screened for chemical-elicited interference in the steroidogenesis pathway, but also identified distinct putative mechanisms of action. For example, few chemicals altered only progestagen levels, while changes in testosterone and estrogen levels were more often observed. These results suggest CYP19A aromatization and CYP17A lyase and hydroxysteroid dehydrogenase activity are more likely targets for the disruption of steroidogenesis by a subset of ToxCast chemicals.

Introduction & Objectives

There are a broad spectrum of environmental chemicals that can disrupt endocrine function resulting in adverse health effects. To-date, endocrine disruption has been largely evaluated as chemical effects on hormone receptor signaling. However, disruption of hormone biosynthesis and metabolism (steroidogenesis) can also constitute endocrine disruption and lead to reproductive and developmental toxicity. Unfortunately, there are currently no established high-throughput *in vitro* models used for the evaluation of chemical-mediated effects on steroidogenesis. The objectives of the current study were:

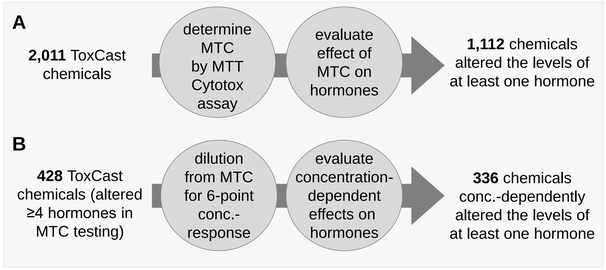
- Establish H295R cells as a high-throughput model for the evaluation of steroidogenesis
- Develop a quantification method allowing for the detection of the 13 major steroid hormones
- Screen the effects of a large diverse library of chemicals on steroidogenesis at concentrations that do not elicit cytotoxicity in concentration-response

Study Design



▲ ***Figure 1. Study design****: H295R human adrenocortical carcinoma cells were pre-stimulated with 10 µM forskolin for 24 h prior to chemical treatment for 48 h, all treatments were conducted in 0.1% DMSO. Media was harvested for hormone quantification by HPLC-MC/MS while cells were tested for viability by MTT assay. The MTT assay was used to ensure ≥80% cell viability for all treatments, dilutions were made if this criteria was not achieved, thus establishing chemical-specific maximum tolerated concentrations (MTC) for this assay.*

Study Workflow and Hormone Quantification

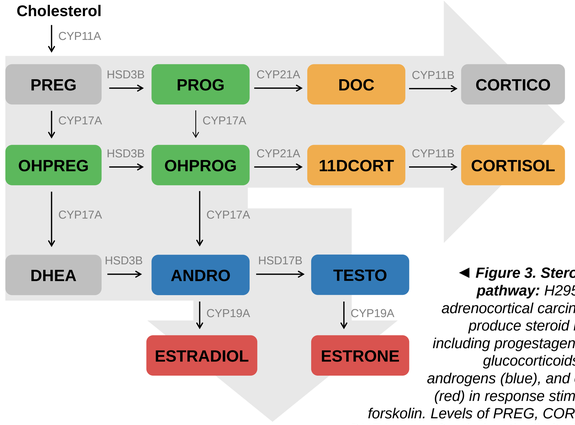


▲***Figure 2. Summary of workflow****: **(A)** Single concentration screening: the maximum tolerated concentration (MTC) for all 2,011 chemicals was identified by MTT assay and evaluated for effects on steroidogenesis. 1,112 chemicals altered at least one hormone. **(B)** Concentration-response evaluation: 428 chemicals that altered ≥4 hormones in the single concentration screening were selected for concentration-response evaluation. 336 chemicals concentration-dependently altered at least one hormone.*

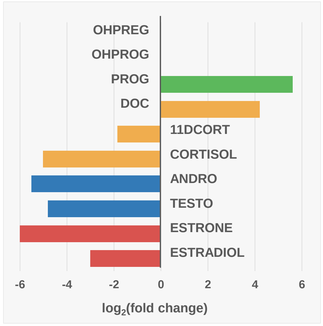
Table 1. Hormones evaluated by HPLC-MS/Ms and Limit of Detection (LOD) ranges per analyte

Hormone	Short Name	LOD (ng/ml)
Pregnenolone	**PREG**	2-400
17α-OH Pregnenolone	**OH-PREG**	5-1000
Progesterone	**PROG**	0.2-40
17α-OH Progesterone	**OH-PROG**	0.2-40
Deoxycorticosterone	**DOC**	0.5-100
Corticosterone	**CORTICO**	0.5-100
11-Deoxycortisol	**11DCORT**	5-1000
Cortisol	**CORTISOL**	0.5-100
Dehydroepiandrosterone	**DHEA**	3-600
Androstenedione	**ANDRO**	1-200
Testosterone	**TESTO**	0.1-20
Estrone	**ESTRONE**	0.03-6
Estradiol	**ESTRADIOL**	0.03-6

Steroidogenesis Pathway and Prochloraz-Mediated Effects



◄ ***Figure 3. Steroidogenic pathway:*** *H295R human adrenocortical carcinoma cells produce steroid hormones including progestagens (green), glucocorticoids (yellow), androgens (blue), and estrogens (red) in response stimulation by forskolin. Levels of PREG, CORTICO and DHEA were often below the limit of detection and omitted from subsequent analyses.*



▲ ***Figure 4. Prochloraz-mediated effects on steroidogenesis:*** *Prochloraz, used as a control chemical as it is known to inhibit CYP17A activity increased PROG and DOC levels while significantly decreasing androgen and estrogen levels. Maximum fold change achieved in concentration-response evaluation is plotted.*

Profiling Chemical Effects on Steroidogenesis

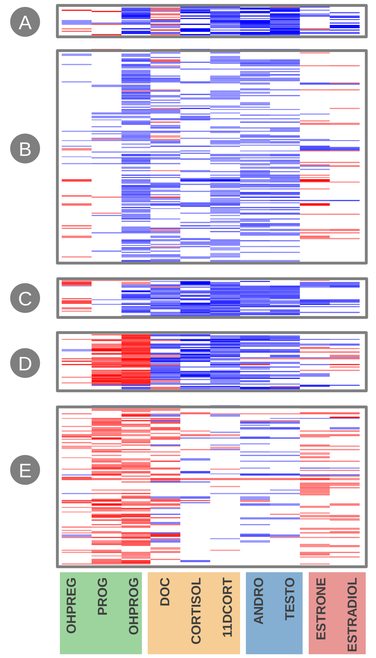


Table 2: K-Means Cluster Profiles

Cluster ID	Effect on Steroidogenesis
A	Overall decrease with increase in DOC
B	Decreased glucocorticoids and androgens
C	Overall decrease with increased OHPREG
D	Increased progestagens with remaining all decreased
E	Increased progestagens and estrogens

◄ ***Figure 5. Clustering of ToxCast Chemical-Mediated Effects on Steroidogenesis:*** *The 336 chemicals that concentration-dependently altered the levels of at least one hormone were clustered using k-means to evaluate profiles of steroidogenesis disruption. White indicates no data or no effect, concentration-dependent increase in hormone levels are indicated in red, concentration-dependent decrease in hormone concentration are shown in blue. Unique profiles including prochloraz-like (cluster A) are discernable.*

Summary

- H295R cells are an ideal high-throughput screening model for evaluating chemical effects on steroidogenesis, with 13 of the major steroid hormones quantifiable via HPLC-MS/MS
- Pre-stimulus with forskolin prior to chemical treatment allows for the detection of both increases and decreases in hormone levels, as demonstrated by prochloraz-mediated inhibition of CYP17A
- 1,112 of the 2,011 chemicals evaluated at a single concentration had effects on at least one hormone
- 336 chemicals concentration-dependently altered the level of at least one hormone
- Distinct profiles of steroidogenesis disruption can be discerned among chemicals, demonstrating the utility of this model to not only identify chemicals that perturb steroidogenesis, but also the ability to evaluate possible mechanisms underlying altered steroidogenesis

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