LCI699 is a Potent Inhibitor of Cortisol Production *in Vitro*

GP26

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Aim

To compare the *in vitro* effects of LCI699, metyrapone, and ketoconazole on cortisol production and steroid hormone profile in human adrenal cells

Introduction

- Ketoconazole and metyrapone are frequently used steroidogenesis inhibitors for treatment of Cushing's syndrome (CS)
- LCI699 (Osilodrostat) is a new steroidogenesis inhibitor which is known to block 11β-hydroxylase
- This compound was originally developed for its inhibitory effects on aldosterone production, but appeared to decrease cortisol response to ACTH-stimulation in patients with hypertension
- LCI699 is now investigated as a potential drug for treatment of CS, but effects on other steroidogenesis enzymes are unknown

Conclusions

- **LCI699 is a potent inhibitor of basal- and ACTH-stimulated cortisol production in adrenocortical tumor cells**
- **LCI699 has only modest inhibitory effects on adrenal androgen production**
- **In these conditions, LCI699 seems to block 11β-hydroxylase (CYP11B1), and to a lesser extent 17,20-lyase activity**
- **The absence of strong accumulation of steroid precursors might indicate an inhibition proximal of 3β-HSD**

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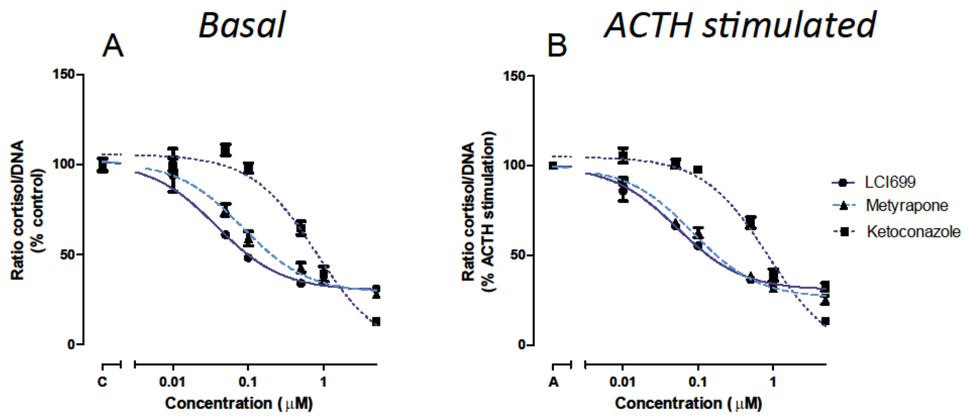
Results

1. Effects of LCI699, metyrapone, and ketoconazole on cortisol production in HAC-15 cells

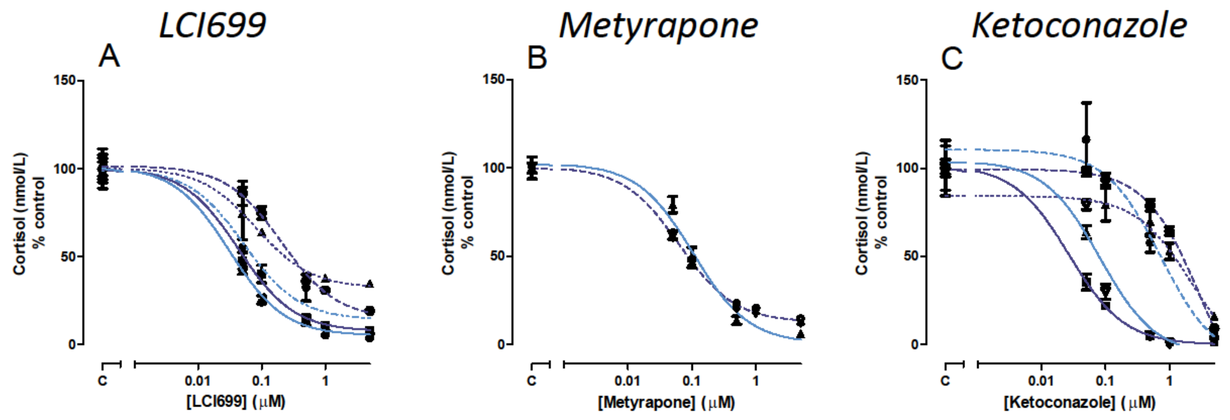


	IC_{50} values	p-value vs LCI699
LCI699	0.038 μM (0.031-0,048)	-
Metyrapone	0.084 μM (0.0602-0.117)	p < 0.0001
Ketoconazole	0.670 μM (0.473-0.950)	p < 0.0001

	IC_{50} values	p-value vs LCI699
LCI699	0.057 μM (0.046-0.070)	-
Metyrapone	0.087 μM (0.067-1.07)	p = 0.0148
Ketoconazole	0.946 μM (0.671-1.333)	p < 0.0001

Figure 1. Effects of LCI699, metyrapone, and ketoconazole on cortisol production in HAC-15 cells under basal conditions (A) and when stimulated with 10 nM ACTH, corrected for cell amount. In the right graph, ACTH stimulation is set as 100%. Cortisol is measured using a chemiluminescence immunoassay system. IC_{50} values represent the half maximal inhibitory concentration. Data are indicated as mean ± SEM. C, control; A, ACTH.

2. Effects of LCI699, metyrapone, and ketoconazole on cortisol production in primary adrenocortical adenoma cultures



	LCI699	Metyrapone	Ketoconazole
Mean IC_{50} value	0.066 μM (0.046-0.096)	0.128 μM (0.0405-0.406)	0.171 μM (0.082-0.36)
p-value vs LCI699	-	p = 0.1197	p = 0.0414

Figure 2. Effects of LCI699 (A), metyrapone (B), and ketoconazole (C) on cortisol production in primary adrenocortical adenoma cultures. Cortisol is measured using a chemiluminescence immunoassay system. Same layout of the lines in the different figures corresponds to the same patient. Mean IC_{50} values represent the mean half maximal inhibitory concentration of all primary cultures. Data are indicated as mean ± SEM. C, control.

3. Effects of LCI699, metyrapone, and ketoconazole on the steroid profile in HAC-15 cells

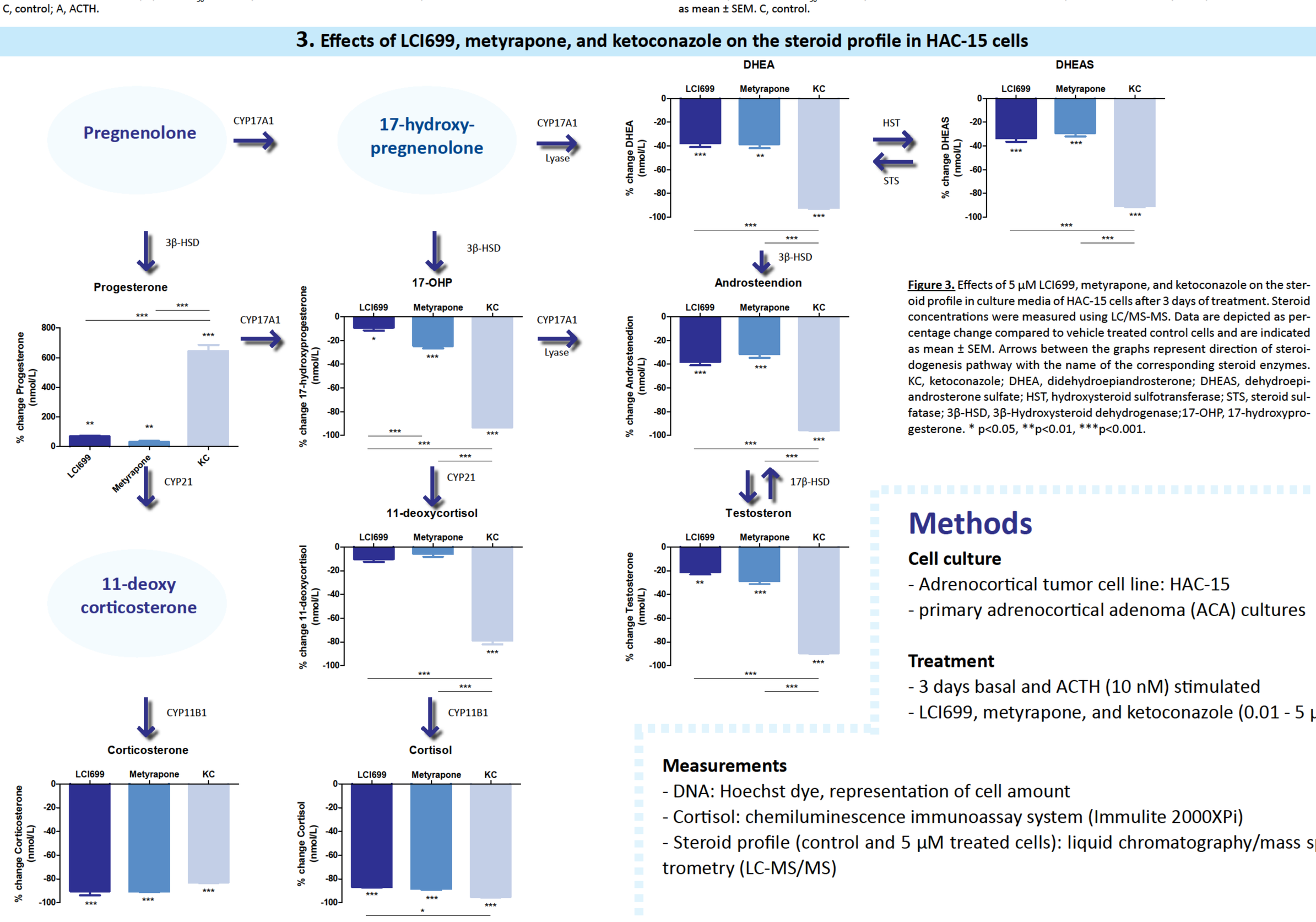


Figure 3. Effects of 5 μM LCI699, metyrapone, and ketoconazole on the steroid profile in culture media of HAC-15 cells after 3 days of treatment. Steroid concentrations were measured using LC/MS-MS. Data are depicted as percentage change compared to vehicle treated control cells and are indicated as mean ± SEM. Arrows between the graphs represent direction of steroidogenesis pathway with the name of the corresponding steroid enzymes. KC, ketoconazole; DHEA, didehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; HST, hydroxysteroid sulfotransferase; STS, steroid sulfatase; 3β-HSD, 3β-Hydroxysteroid dehydrogenase;17-OHP, 17-hydroxyprogesterone. * p<0.05, **p<0.01, ***p<0.001.

Methods

Cell culture
- Adrenocortical tumor cell line: HAC-15
- primary adrenocortical adenoma (ACA) cultures

Treatment
- 3 days basal and ACTH (10 nM) stimulated
- LCI699, metyrapone, and ketoconazole (0.01 - 5 μM)

Measurements
- DNA: Hoechst dye, representation of cell amount
- Cortisol: chemiluminescence immunoassay system (Immulite 2000XPi)
- Steroid profile (control and 5 μM treated cells): liquid chromatography/mass spectrometry (LC-MS/MS)