

SUPPLEMENTARY MATERIAL

Belonging to the manuscript '**Levoketoconazole, the 2S,4R Enantiomer of Ketoconazole, a New Steroidogenesis Inhibitor for Cushing's Syndrome Treatment**'

Supplementary Table 1. Overview of effects of levoketoconazole and racemic ketoconazole on the steroid profile in human adrenocortical cultures.

			Prog	Corticosterone	17-OHP	11-DOC	Cortisol	DHEA	DHEAS	Androstenedione	Testosterone
HAC15	Basal	LK 0.5 μ M	-0.9 \pm 0.1 (-55%)	-0.3 \pm 0.1 (-39%)	-27 \pm 1.7 (-57%)	-1129 \pm 119 (-48%)	-16 \pm 11 (-69%)	-31 \pm 25 (-44%)	-659 \pm 68 (-56%)	-1105 \pm 117 (-56%)	-39 \pm 5.1 (-64%)
		RK 0.5 μ M	-0.3 \pm 0.1 (-19%****)	+0.2 \pm 0.1 (+19%****)	-17 \pm 2.1 (-37%****)	-521 \pm 112 (-23%****)	-11 \pm 0.8 (-47%****)	-40 \pm 2.6 (-56%**)	-496 \pm 38 (-44%*)	-809 \pm 80 (-47%**)	-26 \pm 2.5 (-58%***)
	Effect ACTH	10 nM	+119%	+359%	+35%	+42%	+77%	-35%	-38%	+7%	+7%
	ACTH	LK 0.5 μ M	-2.2 \pm 0.1 (-64%)	-1.1 \pm 0.1 (-36%)	-25 \pm 2.7 (-47%)	-1524 \pm 71 (-47%)	-22 \pm 1.3 (-65%)	-14 \pm 1.8 (-31%)	-250 \pm 34 (-43%)	-944 \pm 79 (-52%)	-31 \pm 2.1 (-71%)
		RK 0.5 μ M	-1.0 \pm 0.1 (-30%****)	-0.0 \pm 0.3 (-1.5%****)	-14 \pm 1.7 (-29%**)	-665 \pm 115 (-22%****)	-15 \pm 1.4 (-45%****)	-17 \pm 2.7 (-41%**)	-197 \pm 22 (-35%)	-661 \pm 67 (-40%*)	-20 \pm 1.4 (-50%****)
ACTH-dependent adrenal hyperplasia no. 1	Basal	LK 0.05 μ M	+5.1 \pm 0.9 (+123%)	-67 \pm 69 (-33%)	-0.5 \pm 1.1 (-4.6%)	-96 \pm 24 (-37%)	-178 \pm 79 (-64%)	< LLQ	< LLQ	-6.5 \pm 1.2 (-64%)	-0.3 \pm 0.1 (-48%)
		RK 0.05 μ M	+4.5 \pm 0.7 (+108%)	-69 \pm 71 (-32%)	+0.7 \pm 0.9 (+7.5%)	-62 \pm 19 (-27%)	-87 \pm 22 (-57%)	< LLQ	< LLQ	-5.9 \pm 1.2 (-55%)	-0.2 \pm 0.1 (-32%)
		LK 5 μ M	+13 \pm 0.5 (+363%)	-194 \pm 48 (-99%)	-14 \pm 0.3 (-93%)	-281 \pm 17 (-99%)	-230 \pm 71 (-99%)	< LLQ	< LLQ	-8.0 \pm 0.8 (-95%)	-0.4 \pm 0.1 (-90%)
		RK 5 μ M	+20 \pm 1.4 (+537%***)	-167 \pm 6.3 (-98%)	-15 \pm 0.8 (-92%)	-271 \pm 18 (-98%)	-165 \pm 16 (-99%)	< LLQ	< LLQ	-8.2 \pm 1.0 (-94%)	-0.3 \pm 0.1 (-66%)
	Effect ACTH	85 pM	-11%	+145%	+94%	+41%	+209%	< LLQ	< LLQ	+64%	+22%
	ACTH	LK 0.05 μ M	+6.0 \pm 0.5 (+167%)	+98 \pm 28 (+29%)	+8.3 \pm 2.8 (+41%)	-6.0 \pm 23 (+1.6%)	-235 \pm 45 (-37%)	< LLQ	< LLQ	-9.6 \pm 1.0 (-50%)	-0.3 \pm 0.1 (-45%)
		RK 0.05 μ M	+3.4 \pm 0.5 (+96%**)	+106 \pm 32 (+31%)	+8.1 \pm 1.1 (+52%)	+77 \pm 23 (+25%**)	-62 \pm 66 (-11%*)	< LLQ	< LLQ	-7.5 \pm 1.5 (-39%)	-0.2 \pm 0.1 (-28%)
		LK 5 μ M	+38 \pm 3.8 (+1201%)	-374 \pm 9.5 (-100%)	-32 \pm 1.6 (-90%)	-435 \pm 14 (-99%)	-640 \pm 12 (-100%)	< LLQ	< LLQ	-15 \pm 0.6 (-98%)	-0.6 \pm 0.1 (-85%)
		RK 5 μ M	+40 \pm 3.3 (+1292%)	-398 \pm 6.7 (-100%)	-28 \pm 1.1 (-90%)	-403 \pm 11 (-99%)	-644 \pm 64 (-100%)	< LLQ	< LLQ	-15 \pm 1.1 (-98%)	-0.4 \pm 0.0 (-85%)
	ACTH-dependent adrenal hyperplasia no. 3	Basal	LK 0.01 μ M	-0.18 \pm 0.18 (-7.7%)	+263 \pm 27 (+88%)	-13 \pm 3.3 (-18%)	-54 \pm 21 (-15%)	-1109 \pm 104 (-37%)	< LLQ	< LLQ	-11 \pm 3.3 (-30%)
RK 0.01 μ M			+0.38 \pm 0.18 (+17%)	+190 \pm 45 (+52%)	-0.8 \pm 3.3 (-0.8%)	-1.8 \pm 17 (-0.5%****)	-431 \pm 140 (-13%****)	< LLQ	< LLQ	-6.4 \pm 2.0 (-18%)	-1.1 \pm 0.32 (-37%)
LK 0.05			+12 \pm 2.9 (+526%)	+347 \pm 42 (+116%)	+42 \pm 9.7 (+59%)	+31 \pm 24 (+9%)	-2421 \pm 129 (-80%)	< LLQ	< LLQ	-29 \pm 3.1 (-81%)	-2.0 \pm 0.098 (-86%)

	μM									
	RK 0.05 μM	+1.1 \pm 0.21 (+49%*)	+475 \pm 62 (+130%)	-3.5 \pm 3.5 (-5.5%****)	+3.1 \pm 19 (+1.0%)	-924 \pm 118 (-28%****)	< LLQ	< LLQ	-16 \pm 2.1 (-46%****)	-1.7 \pm 0.32 (-56%**)
	LK 0.1 μM	+92 \pm 5.7 (+4041%)	-165 \pm 14 (-55%)	+22 \pm 4.8 (+31%)	-209 \pm 18 (-59%)	-2975 \pm 70 (-98%)	< LLQ	< LLQ	-35 \pm 2.9 (-98%)	-2.2 \pm 0.085 (-96%)
	RK 0.1 μM	+12 \pm 1.1 (+532%****)	+517 \pm 45 (+141%****)	+32 \pm 3.1 (+51%)	+92 \pm 15 (+29%****)	-2451 \pm 74 (-73%****)	< LLQ	< LLQ	-27 \pm 1.8 (-77%*)	-2.4 \pm 0.31 (-82%)

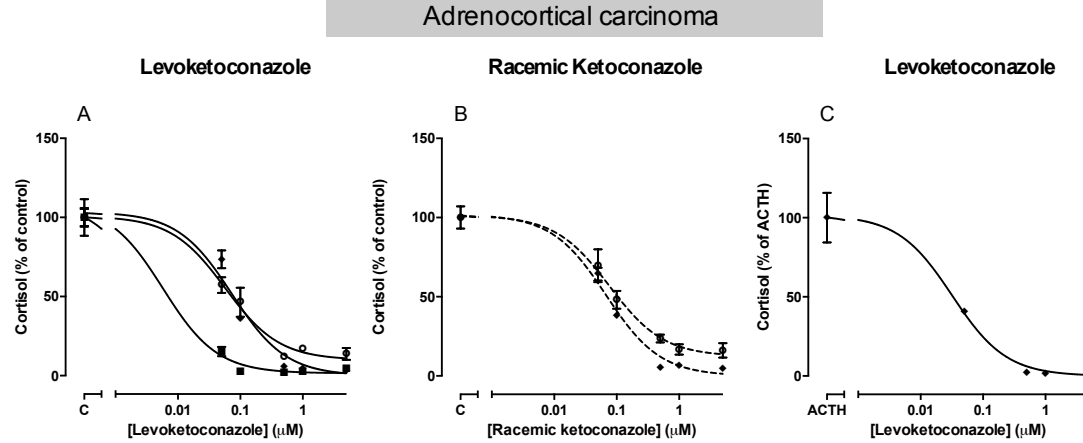
Effects of levoketoconazole (LK) and racemic ketoconazole (RK) on levels of progesterone (prog), corticosterone, 17-hydroxyprogesterone (17-OHP), 11-deoxycortisol (11-DOC), cortisol, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androstenedione, and testosterone after 3 days of treatment. Numbers of the primary cultures correspond to the numbers in Table 1 and 2. Data are presented as absolute change \pm standard error of the difference (in nmol/L) compared to vehicle treated control (basal) or compared to ACTH stimulation with vehicle (ACTH). Significant absolute changes compared to control are depicted in bold. The percentage change compared to control is displayed between brackets. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ compares the percentage change of RK and LK for the same steroid in the same condition in the same culture. ACA, adrenocortical adenoma; ACTH, adrenocorticotrophic hormone; LLQ, lower limit of quantitation.

Supplementary Table 2. Overview of effects of levoketoconazole and racemic ketoconazole on the steroid profile in cortisol-producing ACA no.

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		Prog	Corticosterone	17-OHP	11-DOC	Cortisol	DHEA	DHEAS	Androstenedione	Testosterone
Cortisol-producing ACA no. 2	LK 0.1 μM	+2.4 ± 0.2 (+138%)	+50 ± 6.5 (+54%)	+5.1 ± 0.9 (+26%)	+15 ± 5.8 (+5.4%)	-264 ± 40 (-40%)	< LLQ	< LLQ	-37 ± 1.7 (-79%)	-0.4 ± 0.0 (-66%)
	RK 0.1 μM	+0.5 ± 0.1 (+36%)	+176 ± 5.6 (+213%****)	-0.5 ± 0.6 (-2.4%)	+6.4 ± 9.3 (+2.4%)	-93 ± 40 (-14%**)	< LLQ	< LLQ	-33 ± 2.2 (-66%*)	-0.3 ± 0.0 (-57%)
	LK 0.5 μM	+40 ± 1.8 (+2350%)	+218 ± 3.8 (+239%)	+21 ± 3.9 (+107%)	-98 ± 5.8 (-36%)	-633 ± 25 (-96%)	< LLQ	< LLQ	-45 ± 1.5 (-97%)	-0.5 ± 0.0 (-83%)
	RK 0.5 μM	+22 ± 1.1 (+1567%)	+144 ± 24 (+174%*)	+11 ± 2.0 (+58%*)	-38 ± 17 (-14%***)	-563 ± 40 (-87%)	< LLQ	< LLQ	-47 ± 2.2 (-95%)	-0.4 ± 0.02 (-79%)

Effects of levoketoconazole (LK) and racemic ketoconazole (RK) on levels of progesterone (prog), corticosterone, 17-hydroxyprogesterone (17-OHP), 11-deoxycortisol (11-DOC), cortisol, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androstenedione, and testosterone after 3 days of treatment in cortisol-producing ACA no. 2, corresponding to the number in Table 1 and 2. Data are presented as absolute change ± standard error of the difference (in nmol/L) compared to vehicle treated control. Significant absolute changes compared to control are depicted in bold. The percentage change compared to control is displayed between brackets. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, and **** $P < 0.0001$ compares the percentage change of RK and LK for the same steroid in the same condition in the same culture. ACA, adrenocortical adenoma; ACTH, adrenocorticotrophic hormone; LLQ, lower limit of quantitation.



Supplementary Figure 1. Effects of levoketoconazole (solid lines) and racemic ketoconazole (dotted lines) on cortisol production in primary human adrenocortical carcinoma cultures. The symbols correspond to the symbols as presented in Table 2 and thus correspond to the same patient. Controls represent vehicle treatment without (A, B) or with (C) ACTH stimulation (85 pM). Values are depicted as mean \pm SEM and as percentage of vehicle treated control. ACTH, adrenocorticotrophic hormone; C, control.