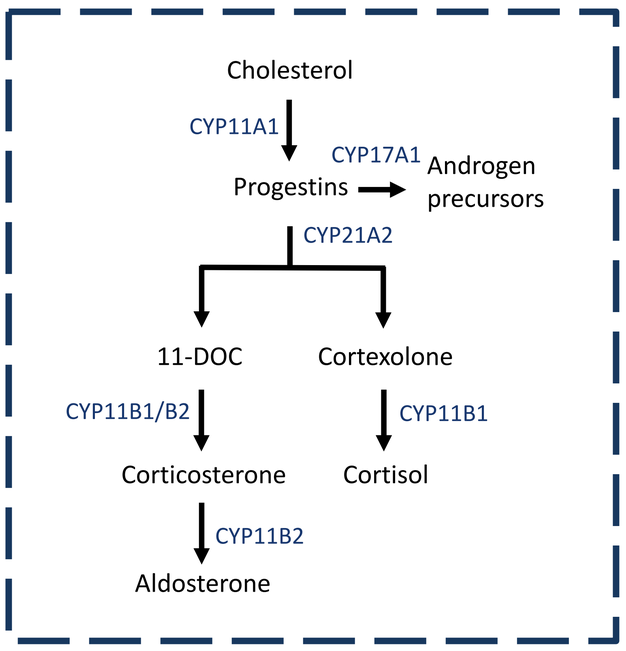
Profiling of azole fungicides for interference with steroidogenesis

Marie-Christin Jäger[1,2], Melanie Patt[1,2], Denise V. Winter[1], Alex Odermatt[1,2]

1. Swiss Centre for Applied Human Toxicology (SCAHT), University of Basel, Basel, Switzerland.
2. Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland.

Background

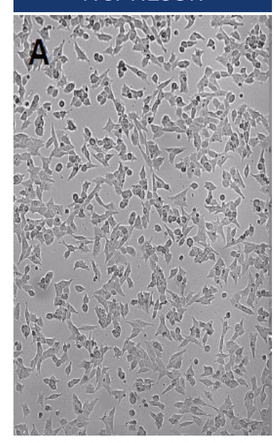
Azole fungicides are used to inhibit fungal CYP51. Many of the approved azole fungicides show adverse effects by modulating human CYP enzymes. Interferences with steroidogenesis can lead to pseudohyperaldosteronism due to the accumulation of mineralocorticoids in response to an unbalanced HPA axis. As a consequence, patients experience secondary hypertension and hypokalemia. To study interferences with steroidogenesis a cell model expressing all key adrenal steroidogenic enzymes was applied.



Methods

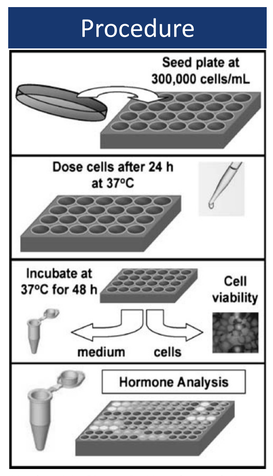
An adapted version of the OECD guideline 456 was used to investigate the extent to which different triazoles affect the concentration of progestins, mineralocorticoids, glucocorticoids and the adrenal androgen DHEA in **human adrenocarcinoma H295R** cells. The cells were incubated with triazoles in a Forskolin stimulated state. After 48 hours the steroid concentration in the supernatant was determined by a targeted ultra-high pressure liquid chromatography-mass spectrometry (**UHPLC-MS/MS**) approach.

NCI-H295R



OECD/OCDE; OECD guideline for the testing of chemicals; H295R Steroidogenesis Assay, OECD 2011

Procedure



Hecker M, Giesy JP; Novel trends in endocrine disruptor testing: the H295R Steroidogenesis Assay for identification of inducers and inhibitors of hormone production. Anal Bioanal Chem. 2008 Jan; 390(1):287-91.

Results

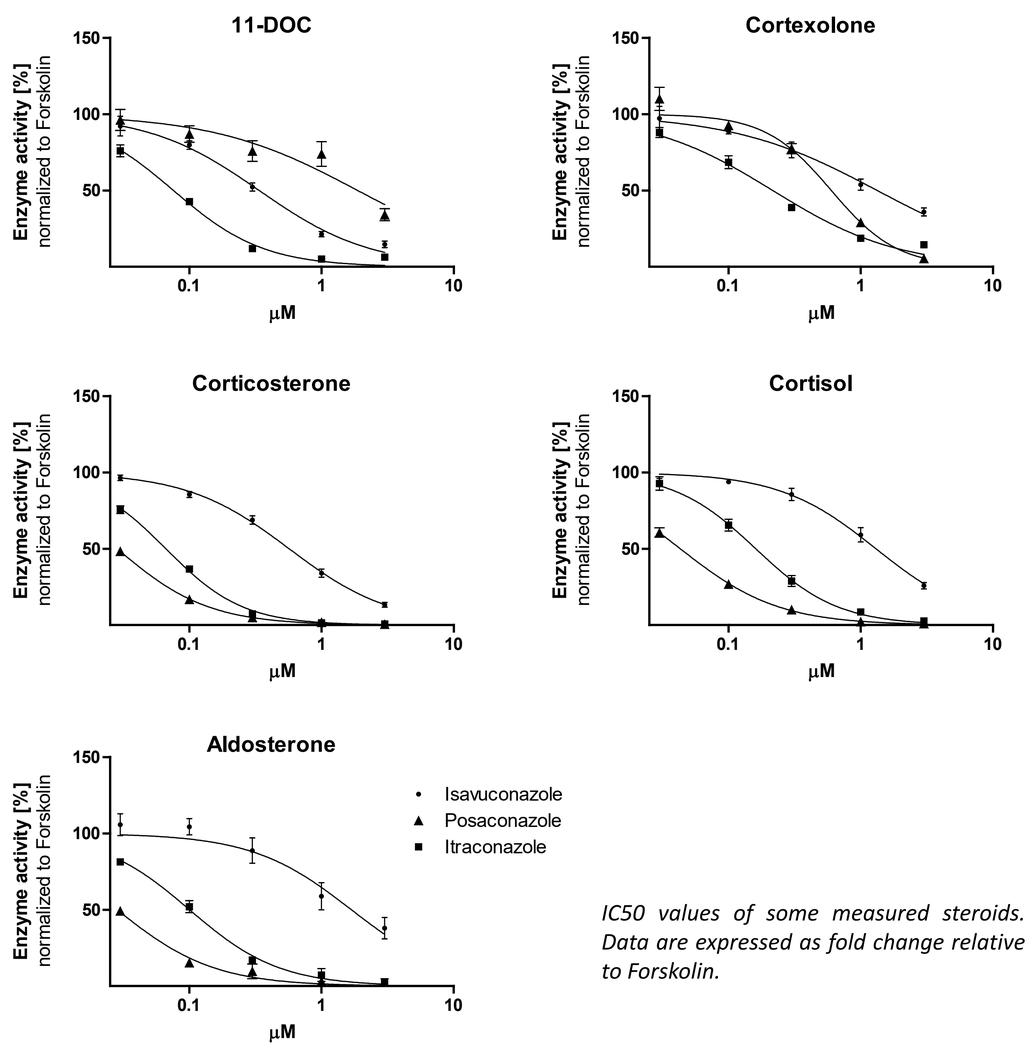
Steroid Profiling in H295R Cells

The results show concentration dependent effects of the triazoles on the steroids. At very high concentrations Isavuconazole reduces mineralocorticoids while glucocorticoids are less affected. Itraconazole reduced the concentration of every steroid measured, with IC values ranging from 90 nM for Progesterone to 500 nM for DHEA. This suggests an inhibition of Cyp11A1 or 3βHSD2. Posaconazole inhibits the production of Aldosterone, Corticosterone and Cortisol with an IC50 of less than 50 nM, suggesting strong inhibition of Cyp11B1 and Cyp11B2. Voriconazole and Fluconazole showed no strong inhibitory effects on the tested steroids.

		progestins		mineralocorticoids			glucocorticoids		adrenal androgens
	treatment \ hormone	progesterone	17α-hydroxy-progesterone	11-deoxy-corticosterone	corticosterone	aldosterone	cortexolone	cortisol	dehydro-epiandrosterone
FC	forskolin 10 µM	1.01 ± 0.0544	1.001 ± 0.02873	1 ± 0.0453	0.998 ± 0.0321	1.004 ± 0.018	1 ± 0.029	1.006 ± 0.0271	0.9982 ± 0.1584
MC	complete medium; t=0	0.17 ± 0.0482	n.d.	n.d.	n.d.	n.d.	0.004 ± 0.002	0.00839 ± 0.0111	n.d.
Azole fungicides	Prochloraz 1 µM	23.2 ± 2.84	2.091 ± 0.3277	3.016 ± 0.6528	0.183 ± 0.0475	0.352 ± 0.051	0.184 ± 0.043	0.01789 ± 0.0092	n.d.
	Posaconazole 3 µM	1.04 ± 0.0812	0.09048 ± 0.02023	0.3415 ± 0.065	0.006 ± 0.0093	n.d.	0.055 ± 0.003	0.01015 ± 0.0092	n.d.
	Posaconazole 1 µM	0.96 ± 0.0887	0.2121 ± 0.03984	0.7711 ± 0.1098	0.015 ± 0.0125	n.d.	0.296 ± 0.025	0.02 ± 0.0079	n.d.
	Posaconazole 0.3 µM	0.69 ± 0.0942	0.501 ± 0.09536	0.7584 ± 0.1065	0.05 ± 0.0152	n.d.	0.774 ± 0.063	0.1009 ± 0.0159	0.4379 ± 0.03537
	Posaconazole 0.1 µM	0.78 ± 0.097	0.7595 ± 0.07394	0.8695 ± 0.0974	0.169 ± 0.0261	n.d.	0.925 ± 0.047	0.268 ± 0.0328	0.8239 ± 0.0663
	Posaconazole 0.03 µM	0.88 ± 0.0945	0.8942 ± 0.07839	0.9622 ± 0.1152	0.483 ± 0.029	0.492 ± 0.051	1.1 ± 0.15	0.606 ± 0.0494	0.9823 ± 0.09493
	Itraconazole 3 µM	0.13 ± 0.0218	0.0863 ± 0.01905	0.0633 ± 0.0078	0.007 ± 0.0115	n.d.	0.145 ± 0.016	0.02662 ± 0.0143	0.1628 ± 0.02986
	Itraconazole 1 µM	0.1 ± 0.0205	0.06697 ± 0.01585	0.0505 ± 0.0073	0.015 ± 0.0148	n.d.	0.188 ± 0.019	0.08563 ± 0.0097	0.1943 ± 0.0327
	Itraconazole 0.3 µM	0.18 ± 0.014	0.1975 ± 0.03421	0.1198 ± 0.0117	0.074 ± 0.0144	n.d.	0.388 ± 0.03	0.289 ± 0.0552	0.6267 ± 0.09883
	Itraconazole 0.1 µM	0.49 ± 0.0508	0.5719 ± 0.05997	0.4271 ± 0.0155	0.368 ± 0.0325	0.52 ± 0.064	0.685 ± 0.065	0.6544 ± 0.0608	0.9328 ± 0.09598
	Itraconazole 0.03 µM	0.77 ± 0.0854	0.7839 ± 0.07096	0.7596 ± 0.0811	0.756 ± 0.045	0.813 ± 0.04	0.88 ± 0.077	0.9276 ± 0.0777	1.03 ± 0.08509
	Isavuconazole 3 µM	0.17 ± 0.0788	0.1713 ± 0.03856	0.15 ± 0.0329	0.133 ± 0.025	n.d.	0.359 ± 0.043	0.2578 ± 0.0326	0.3103 ± 0.04542
	Isavuconazole 1 µM	0.23 ± 0.0833	0.2823 ± 0.04899	0.2136 ± 0.0329	0.34 ± 0.05	0.588 ± 0.146	0.538 ± 0.061	0.591 ± 0.0846	0.5399 ± 0.1225
	Isavuconazole 0.3 µM	0.54 ± 0.029	0.5953 ± 0.02381	0.5227 ± 0.0411	0.689 ± 0.0492	0.888 ± 0.132	0.765 ± 0.086	0.8558 ± 0.0657	0.8557 ± 0.09576
	Isavuconazole 0.1 µM	0.79 ± 0.0491	0.8046 ± 0.04475	0.7978 ± 0.0511	0.855 ± 0.0403	1.044 ± 0.085	0.888 ± 0.031	0.9387 ± 0.0394	0.942 ± 0.1168
	Isavuconazole 0.03 µM	0.91 ± 0.098	0.8685 ± 0.07855	0.9213 ± 0.1062	0.935 ± 0.0482	1.029 ± 0.113	0.972 ± 0.149	0.9365 ± 0.0402	1.02 ± 0.0975
	Voriconazole 3 µM	0.7 ± 0.0897	0.7526 ± 0.08111	0.6344 ± 0.0333	0.588 ± 0.0201	0.737 ± 0.067	0.868 ± 0.108	0.6907 ± 0.0677	0.8001 ± 0.0978
	Voriconazole 1 µM	0.79 ± 0.0676	0.8259 ± 0.04762	0.7975 ± 0.1017	0.839 ± 0.0285	0.961 ± 0.077	0.967 ± 0.131	0.8701 ± 0.0695	0.9257 ± 0.08594
	Fluconazole 3 µM	0.99 ± 0.1414	0.9195 ± 0.1155	1.038 ± 0.0901	0.758 ± 0.0851	0.99 ± 0.105	1.057 ± 0.165	0.7515 ± 0.1624	1.042 ± 0.1419
	Fluconazole 1 µM	0.91 ± 0.1574	0.8822 ± 0.09795	0.993 ± 0.084	0.938 ± 0.029	1.042 ± 0.131	1.004 ± 0.094	0.9221 ± 0.0546	1.177 ± 0.1127

Quantitative analysis of effects of test compounds on the H295R profile. Data are expressed as fold-changes relative to Forskolin treatment (mean ± SD; n = 6). Steroid metabolites significantly down regulated are indicated in green, upregulated in red (p-value < 0.05). FC means forskolin control, MC means medium control, nd means not detected,

IC50 of steroid production



IC50 values of some measured steroids. Data are expressed as fold change relative to Forskolin.

Conclusion

The chosen approach provided novel mechanistic insight into the inhibition of steroidogenesis by selected triazoles. Posaconazole and Itraconazole exhibit different inhibitory actions. Voriconazole and Fluconazole showed no strong inhibitory actions. The differential effects of Isavuconazole, Posaconazole and Itraconazole will need to be further investigated in vivo as well as using individual enzyme assays.

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