Species-specific differences in the inhibition of 11β-hydroxysteroid dehydrogenase 2 by itraconazole

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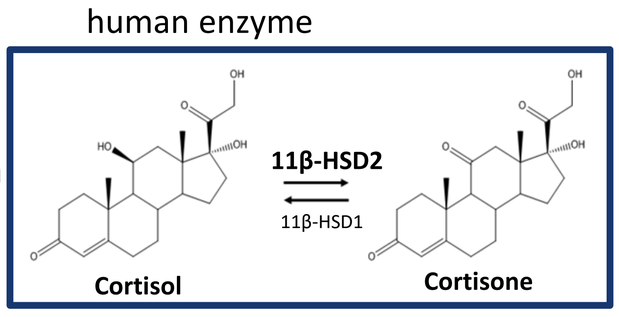
Background

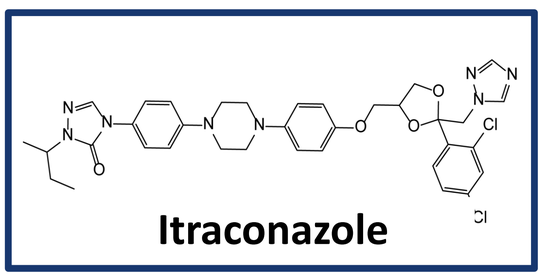
11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts active 11β-hydroxyglucocorticoids (**cortisol, corticosterone)** in their inactive 11-keto forms (**cortisone**, 11-dehydrocorticosterone), thus preventing inappropriate mineralocorticoid receptor activation by glucocorticoids. Disruption of 11β-HSD2 activity by genetic defects or potent inhibitors including azole antifungal **itraconazole** cause the syndrome of **apparent mineralocorticoid excess** (AME), characterized by hypokalemia, hypernatremia and hypertension.

To determine why this adverse drug effect was missed during preclinical investigations, the inhibitory potential of itraconazole against 11β-HSD2 from human and three commonly used experimental animals was assessed.

Methods

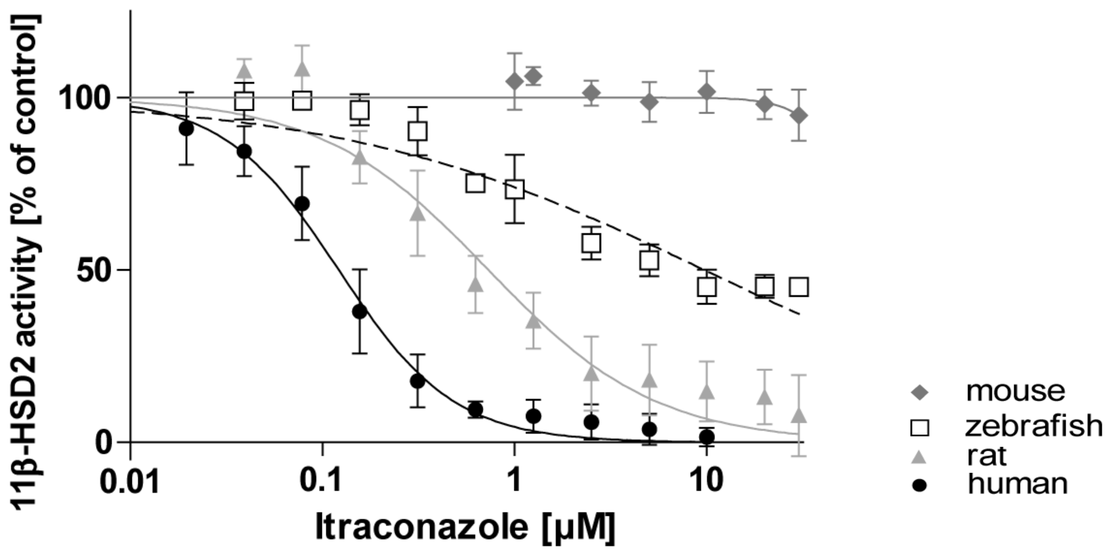
- IC_{50} determination of itraconazole in human, mouse, rat and zebrafish 11ß-HSD2 by cell free enzyme activity assays
- Sequence alignment and homology modeling comparing human and mouse 11ß-HSD2
- Molecular cloning to exchange previously identified structurally different C-termini and substitution of residues Leu170,Ile172 in mouse 11β-HSD2 by the corresponding residues His170,Glu172 of the human enzyme





Results

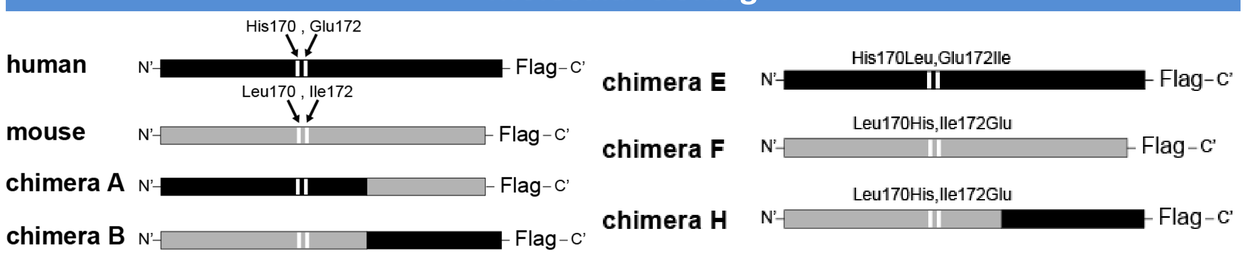
Species-specific differences in the inhibition of 11β-HSD2 by itraconazole



Inhibition of 11β-HSD2 activity by itraconazole: IC_{50} [µM]			
Human	Mouse	Rat	Zebrafish
0.121 ± 0.030	(95%)*	0.729 ± 0.592	9.80 ± 1.96

IC_{50} values for human, mouse, rat and zebrafish 11β-HSD2 of itraconazole. Determination of 11β-HSD2 activity of different species in presence of increasing concentrations of itraconazole revealed potent inhibition of human 11ß-HSD2, moderate inhibition of rat enzyme (6-fold higher IC_{50} value compared to human), and weak inhibition of mouse and zebrafish 11β-HSD2 (IC_{50} values above 7 µM). *% remaining activity at the highest concentration of 30 µM.

Molecular cloning

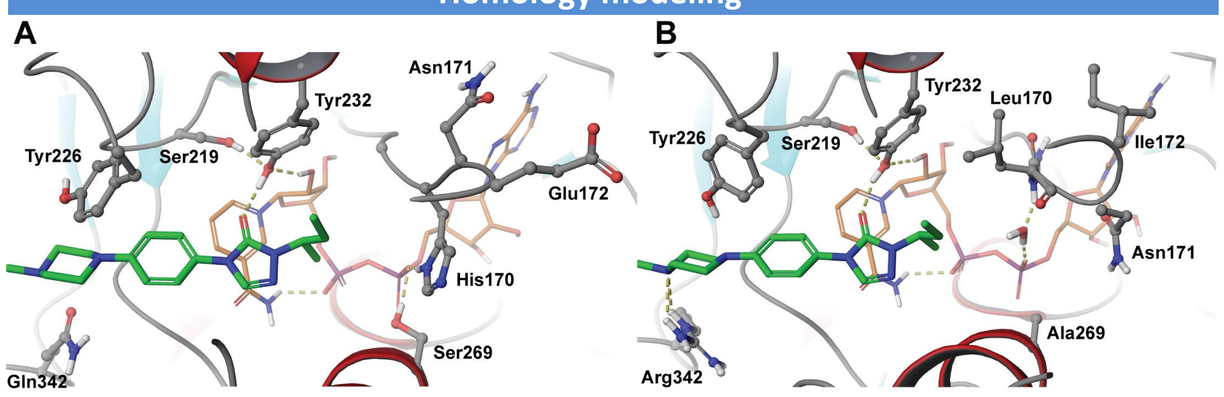


Generation of human-mouse 11ß-HSD2 chimera by exchanging C-termini and residues 170,172 previously identified by homology modeling. Exchange of the C-terminus and substitution of residues Leu170,Ile172 in mouse 11β-HSD2 by the corresponding residues His170,Glu172 of the human enzyme resulted in a gain of sensitivity to itraconazole, resembling human 11β-HSD2.

Sequence alignment

Sequence alignment identified the largest differences to be in the C-terminus between human and mouse 11ß-HSD2

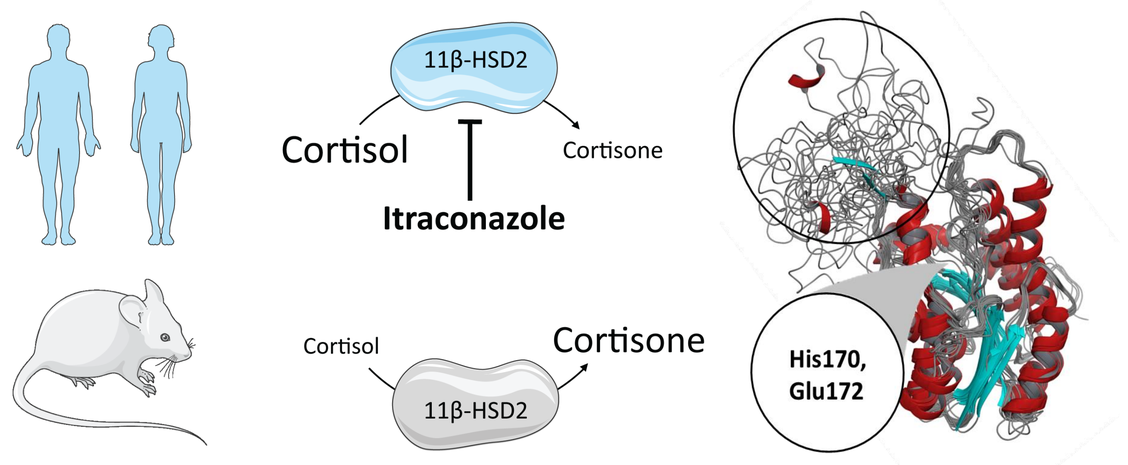
Homology modeling



Itraconazole (green) and NAD+ (orange) docked into A) human and B) mouse 11β-HSD2 homology models. A closer look in human 11β-HSD2 on the binding site region, indicated that the loop portion around histidine residue 170 (His170) could be responsible for interspecies differences in the enzyme selectivity. The construction of this new homology model showed that His170 offers an ideal hydrogen bonding interaction to the cortisol C20 carbonyl. Even though Glu172 is not directly involved in substrate binding, it was proposed to interact with Leu179, which is if mutated to Arg179 a known genetic cause of AME. (Yau et al., 2017).

Conclusion

Human C-terminus and His170,Glu172 render mouse 11β-HSD2 sensitive to itraconazole



The gained structural understanding will facilitate future investigations of 11β-HSD2 inhibitors

Knowledge of species differences may aid in choosing suitable animal models to investigate 11β-HSD2 inhibitors

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