Model systems to define the role of AKR1D1 in metabolic liver disease

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Background

Non-alcoholic fatty liver disease is the hepatic manifestation of the global epidemic of metabolic disease. It is tightly associated with obesity and type 2 diabetes, yet the precise mechanisms that drive its aetiology are not fully defined. Steroid hormones, including glucocorticoids and sex steroids, regulate metabolic phenotype; additionally, bile acids have recently been identified as potent metabolic regulators. AKR1D1 (5β-reductase), is predominantly expressed in the liver, and is a crucial regulator of steroid hormone clearance as well as bile acid synthesis. Its role in pathogenesis of metabolic disease has not been examined. We therefore developed systems to define the enzymology of human AKR1D1 in cell free assays and to determine the impact of manipulation of AKR1D1 expression and activity in human hepatocyte models.

Methods

B21 Rosetta bacteria cells were transformed with an AKR1D1 construct (pNIC-CTHF+AKR1D1) and recombinant protein extracted and purified. A high throughput assay was developed to determine AKR1D1 activity, substrate specificity and enzyme kinetics. 9 different substrates (7 steroids and 2 bile acid intermediates) were tested against the purified enzyme and activity was measured by NADPH reduction in a dose and time dependent manner. AKR1D1 mRNA expression was characterized in 4 different hepatoma cell lines (Hep3b, HepG2, C3A and Huh7.0) as well as primary cultures of human hepatocytes. In addition, HepG2 cells were differentiated using an established protocol (including 1% DMSO treatment), and gene expression analyzed after 7, 14 and 21 days. Over-expression and siRNA knock down of AKR1D1 in HepG2 cells were also performed. AKR1D1 was highly expressed in HepG2 cells and expression decreased across differentiation, to levels that were similar to those seen in primary cultures of human hepatocytes. Successful over-expression and knock down of AKR1D1 were confirmed in HepG2 cells using real-time PCR. Importantly, changes in gene expression were paralleled by functional activity as measured by progesterone clearance.

Results

1. Substrate specificity of AKR1D1

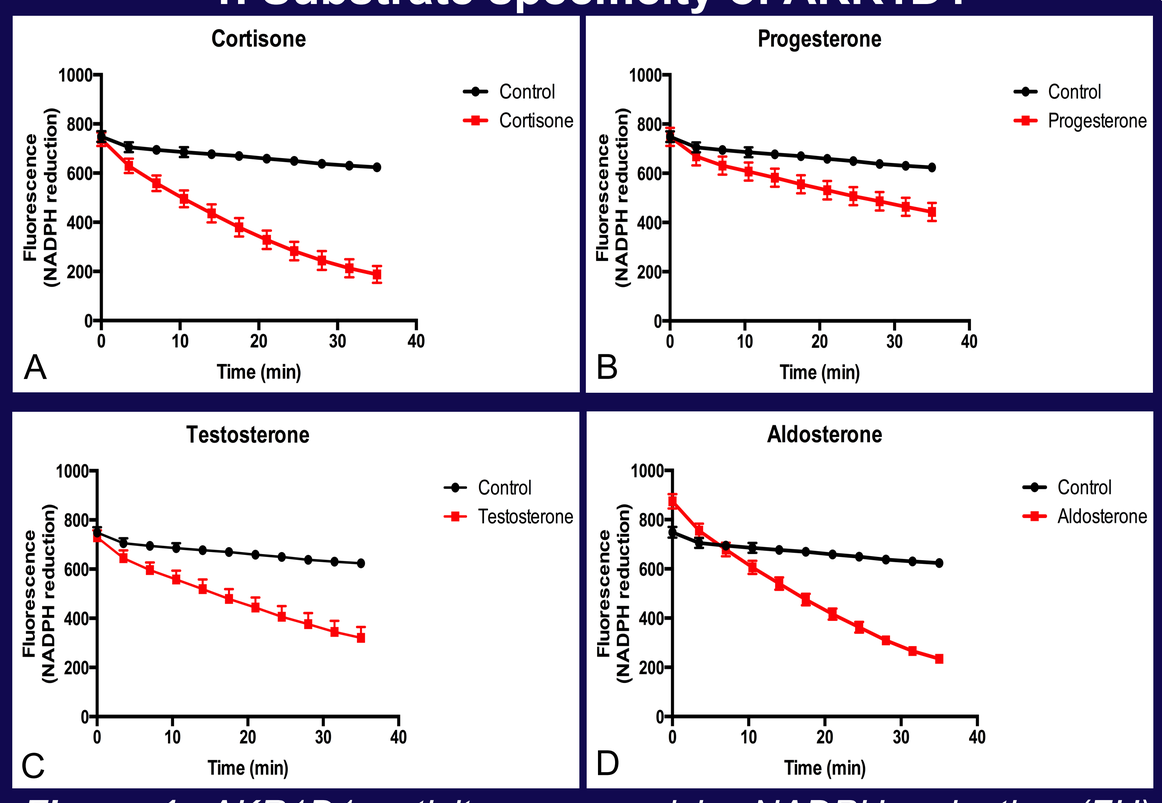

Figure 1: *AKR1D1 activity measured by NADPH reduction (FU) using Cortisone (A), Progesterone (B), Testosterone (C) and Aldosterone (D) as a substrate.*

2. Enzyme kinetics of AKR1D1

Substrate	Km	Kcat	Kcat/Km
Androstenedione	3.1	0.35	0.113
Progesterone	3.29	0.25	0.077
Corticosterone	4.36	0.30	0.069
Cortisone	5.44	0.73	0.134
Aldosterone	6.65	0.83	0.124
Testosterone	7.2	0.73	0.101
7a,12a dihydroxy cholestenone	15.82	0.15	0.009
Cortisol	25.42	0.46	0.018
7a hydroxy cholestenone	25.77	0.24	0.009

Figure 2: *Km, Kcat and Kcat/Km values for all AKR1D1 substrates as measured by high throughput screening assay.*

3. AKR1D1 inhibition by 5αR inhibitors

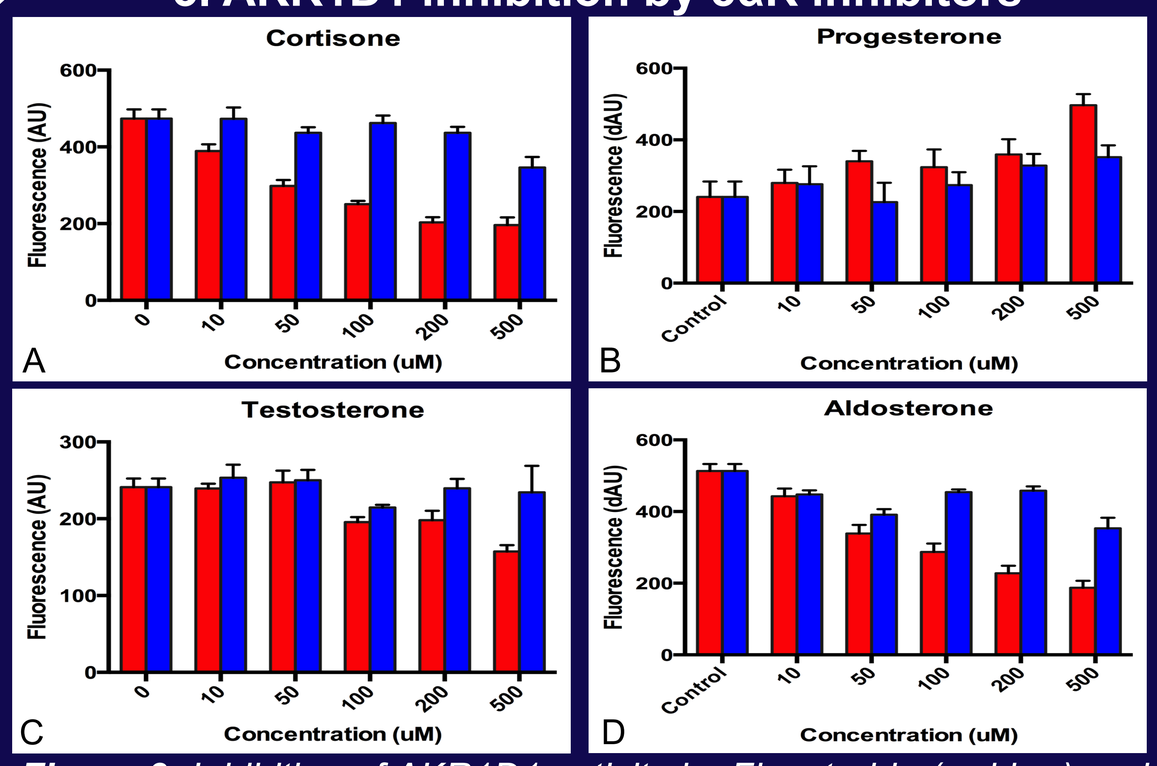

Figure 3*: Inhibition of AKR1D1 activity by Finasteride (red bar) and Dutasteride (blue bar) using Cortisone (A), Progesterone (B), Testosterone (C) and Aldosterone (D) as a substrate, measured by NADPH reduction (FU).*

4. Genetic manipulation of AKR1D1

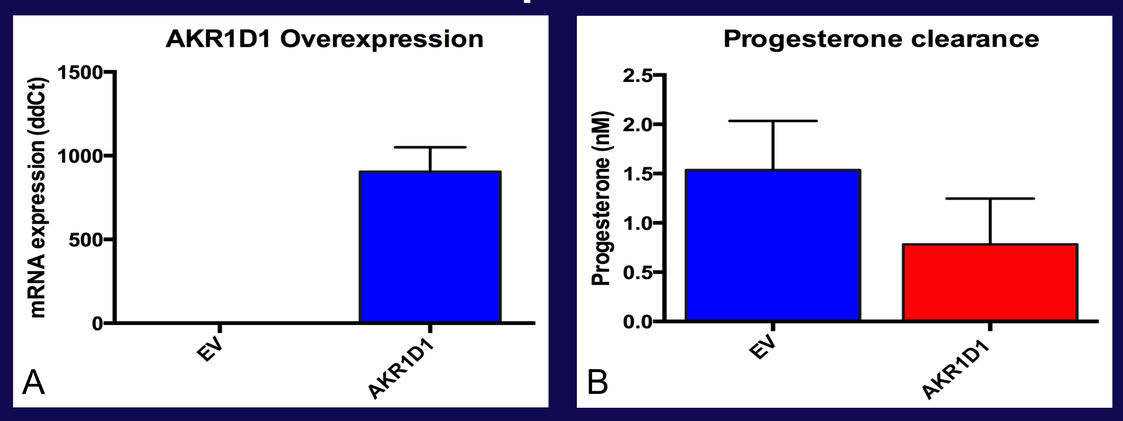

Figure 4: *AKR1D1 overexpression in HepG2 cells, as measured by real-time PCR (A) and progesterone clearance (B).*

5. HepG2 differentiation by DMSO treatment

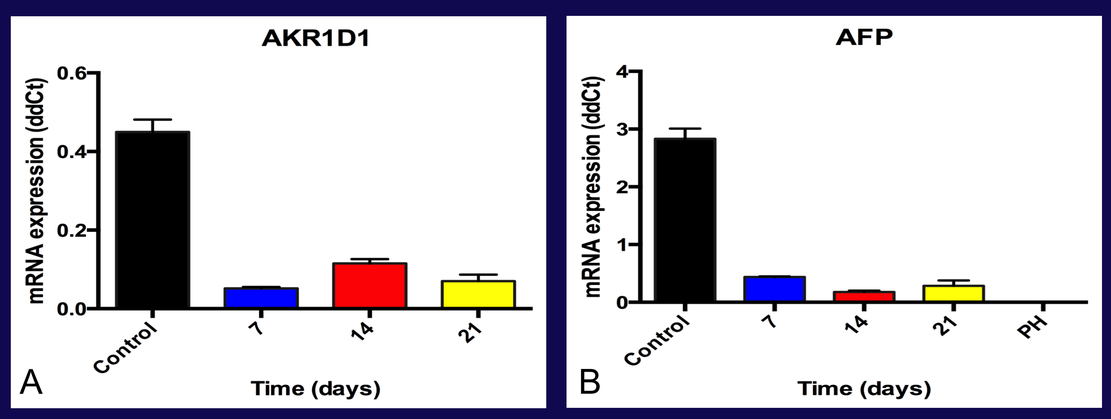

Figure 5: *AKR1D1 (A) and AFP (B) mRNA expression after differentiation with 1% DMSO as measured by real-time PCR.*

Conclusion

We have successfully manipulated AKR1D1 expression and activity that will serve as the platform for future studies to define its role in the regulation of metabolic phenotype within the liver.