

11 β -HYDROXYSTEROID DEHYDROGENASE 1: A MULTI-FUNCTIONAL ENZYME

Safety Assessment of Inhibitors



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Introduction

11 β -hydroxysteroid dehydrogenase 1 (11 β -HSD1) converts inert 11-ketoglucocorticoids (cortisone, 11-dehydrocorticosterone) into active 11 β -hydroxyglucocorticoids (cortisol, corticosterone) and is mainly expressed in liver, adipose tissue and brain (Fig. 1).

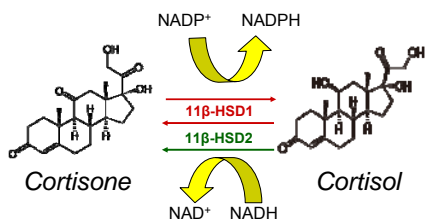


Fig. 1: Glucocorticoid metabolism by 11 β -HSDs

11 β -HSD1 as a drug target

Transgenic mice overexpressing 11 β -HSD1 selectively in adipose tissue develop metabolic syndrome with visceral obesity, impaired insulin-stimulated glucose disposal, dyslipidemia and hypertension. In obese humans and rodents 11 β -HSD1 expression is elevated in adipose tissue. In contrast, 11 β -HSD1 knock-out mice have a cardioprotective phenotype and are resistant to high-fat diet induced obesity and insulin resistance.

11 β -HSD1 overexpression in adipose tissue

- Visceral obesity
- Impaired insulin-stimulated glucose disposal
- Dyslipidemia
- Hypertension

11 β -HSD1 knock-out mice

- Cardioprotective phenotype.
- Resistance to high-fat diet induced obesity.
- Insulin sensitivity.
- Glucose tolerance.

Inhibition of 11 β -HSD1 is considered a promising strategy to treat patients with metabolic syndrome by reducing glucocorticoid levels in adipose tissue, liver and skeletal muscle. Currently, several inhibitors are used in ongoing clinical trials.

Functions of 11 β -HSD1

11 β -HSD1 is a promiscuous enzyme, playing a role in several biochemical processes. For safety assessment of 11 β -HSD1 inhibitors, these so-called alternative functions need to be investigated and sensitive methods are required to detect potential side-effects upon inhibition of this enzyme.

Reduction of xenobiotics:
metirapone, triadimefene, NNK, others

Metabolism of endogenous sterols:
7-ketocholesterol

Metabolism of neurosteroids:
7- α -DHEA, 7- α -pregnenolone, 11- α -oxyprogesterone, 11- α -oxyandrogens

Metabolism of bile acids:
7-keto-lithocholic acid

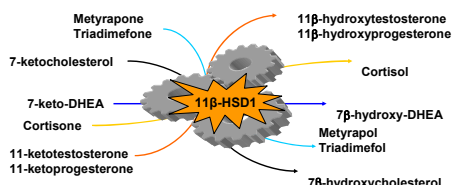


Fig. 2: 11 β -HSD1 metabolizes xenobiotics and endogenous compounds

11 β -HSD1: role in bile acid metabolism

We tested the hypothesis that 11 β -HSD1 might play a role in bile acid metabolism and by converting (Fig. 3).

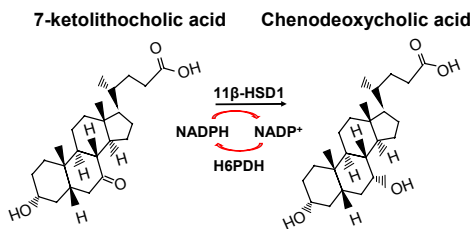
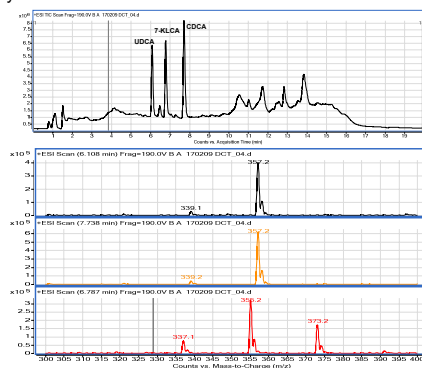


Fig. 4: Qualitative profile of CDCA, UDCA and 7KLCA by LC-MS/MS



7-ketolithocholic acid metabolism by 11 β -HSD1

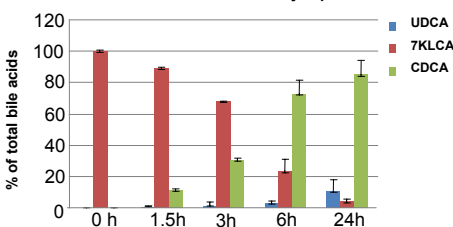
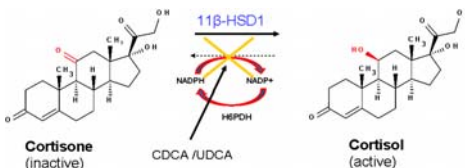


Fig. 5: Intact HEK-293 cells stably transfected with 11 β -HSD1 and H6PDH were incubated with 7-ketolithocholic acid (1 μ M). At different time points, the formation of CDCA and UDCA from 7KLCA was detected and quantified by LC-MS.

Inhibition of 11 β -HSD1 dehydrogenase and reductase activity by 7-oxybile acids

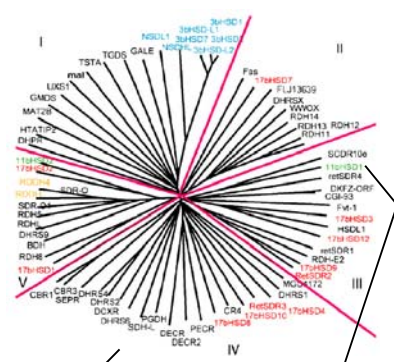


	Reductase	Dehydrogenase
	Cortisone $\xrightarrow{11\beta\text{-HSD1}}$ Cortisol	Cortisol $\xrightarrow{11\beta\text{-HSD1}}$ Cortisone
7-ketolithocholic acid (7-KLCA)	IC ₅₀ 1.07 \pm 0.26	IC ₅₀ 2.77 \pm 0.81
Chenodeoxycholic acid (CDCA)	IC ₅₀ 4.1 \pm 0.6	IC ₅₀ 0.27 \pm 0.08

Table 1: 7-ketolithocholic acid and chenodeoxycholic acid were used as inhibitors in the conversion of cortisone to cortisol and the reverse reaction.

LC-MS/MS-based metabolomics

Fig. 7. Short-chain reductase/dehydrogenase super family



Off target inhibition

On target inhibition

Profiling metabolites/steroids using LC-MS/MS QQQ. (qualitative and quantitative analysis)

Steroids, sterols, prostaglandins, bile acids, retinoids, fatty acids – early detection of altered concentrations in plasma, tissues and urine.

Outlook

We demonstrate a novel function of 11 β -HSD1 in the metabolism of 7-ketolithocholic acid. The conversion of this oxidized bile acid that is mainly formed by microbial enzymes in the gut has been reported more than 20 years ago. However, the enzyme responsible in the liver for the conversion of 7-ketolithocholic acid to chenodeoxycholic acid remained unknown so far.

Our results provide further evidence for the multi-functionality of 11 β -HSD1. It is important to understand the relevance of the so-called alternative functions of this enzyme with regard to the development of inhibitors for the treatment of patients with metabolic disease.

Regarding the low circulating concentrations of 7-ketolithocholic acid an inhibition of 11 β -HSD1 by this bile acid seems unlikely. However, whether inhibition will lead to disturbances in bile acid dependent functions remains to be investigated.

Future studies are required to address potential side-effects of 11 β -HSD1 inhibition with respect to its role in the so-called alternative functions.

The implementation of LC-MS/MS will allow to monitor changes in the conversion of endogenous and exogenous substrates *in vivo*. Furthermore, potential side-effects due to the inhibition of other SDR enzymes might be detectable at an early stage.

Acknowledgements

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