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Abstract

Towards enzyme replacement therapy as a treatment for SSADH-deficiency

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Abstract: Succinic semialdehyde dehydrogenase deficiency (SSADH-D) is a rare monogenic disorder of the γ-amino butyric acid (GABA) metabolism. Various pathogenic mutations in aldehyde dehydrogenase 5 family member A1 (ALDH5A1) gene are responsible for the enzymatic dysfunction of the succinic semialdehyde dehydrogenase (SSADH), an enzyme that plays a key role in the breakdown of GABA. As a consequence, GABA and its potentially toxic metabolite γ–hydroxybutyrate (GHB) accumulate in the brain and physiological fluids. The aim of this study was to produce and test different recombinant SSADH proteins for an enzyme-replacement therapy for SSADH-D. The intracellular delivery of large bioactive molecules, such as enzymes, requires that these molecules traverse not only the plasma membrane, but also further intracellular membranes. Thus, a cell-penetrating peptide (Trans-activator of Transcription; Tat) was fused to the N-terminal part of SSADH. This sequence was followed by mitochondrial targeting sequence (MTS), as SSADH is a mitochondrial enzyme (rHis-Tat-MTS-SSADH). The sequence of human SSADH as well as MTS and Tat were optimized for efficient bacterial overexpression. As a control, optimized sequences lacking MTS and Tat were produced either with (rHis-SSADH) or without His-tag (rSSADH). In-vitro, purified rHis-SSADH and rSSADH, but not in rHis-Tat-MTS-SSADH, exhibited SSADH activity. Interestingly, all produced recombinant enzymes displayed a highly efficient cellular and mitochondrial uptake in SSADH-D patient fibroblasts. However, only rHis-SSADH and rSSADH were able to fully reconstitute the missing SSADH activity. These effects were His-independent. Although rHis-Tat-MTS-SSADH reached the mitochondrial compartment, it was not processed in the mature form and thus showed no SSADH activity. These results indicate that rHis-SSADH and rSSADH are suitable candidates for further testing in an animal model for SSADH-D.