Lentiviral vector EAAT2 infected human astrocyte cultures show an increased glutamate uptake

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Amyotrophic lateral sclerosis (ALS) is a neurological disorder in which motoneurons degenerate, causing paralysis and eventually death after 2-5 years. There is ample evidence that glutamate excitotoxicity is critically involved in ALS pathogenesis. The glutamate transporter EAAT2, localized on astrocytes, normally removes glutamate from the extracellular milieu to protect motoneurons from excitotoxic cell death. In ALS patients EAAT2 protein expression and function is dramatically decreased. Interestingly, in an EAAT2/G93A-hSOD double transgenic ALS mouse model, it has been shown that EAAT2 overexpression partially prevents motoneuron degeneration and delays the onset of disease. To compensate for the loss of EAAT2 in ALS, and, hence, to protect motoneurons from excitotoxicity, we postulate that lentiviral gene delivery of EAAT2 into the spinal cord might be of therapeutic value. Here, we investigate whether a LVV-EAAT2 construct is able to transduce primary human astrocytes, isolated either from human post-mortem spinal cord or cortex. Human astrocyte cultures were characterized by immunocytochemistry for the glial fibrillary acidic protein (GFAP), a specific marker for astrocytes. After transfection, more than 98% of the cells stained positive for GFAP. Increased EAAT2 protein level in LVV-EAAT2 infected cells was shown by Western blot analysis while the functionality of the transduced EAAT2 was examined by a [^{3}H]glutamate uptake assay. To construct a Lineweaver-Burk plot (to determine V_{max} and K_m), uptake assays were performed with different concentrations of glutamate and a constant amount of [^{3}H]glutamate. It was found that human astrocytes transduced with LVV-EAAT2 showed a statistically significant increase in glutamate uptake compared to both uninfected and LVV-green fluorescent protein (GFP) transduced control cells. From the outcome of this in vitro study, it is concluded that direct lentiviral transfer of EAAT2 to spinal cord astrocytes is feasible and might be considered as therapeutic option in ALS.

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