An inhibited dopamine synthesizing cell model of AADC deficiency

ABSTRACT

Introduction: Aromatic L-amino acid decarboxylase deficiency (AADC) is a rare autosomal recessive pediatric neurotransmitter disease. To date it remains poorly understood mainly due to an absence of a disease model. The dopaminergic neuroblastoma cell SH-SY5Y was chosen to develop our AADC deficiency model. These cells are not native dopamine synthesizers. Objective: To develop a dopamine-producing cellular model of AADC deficiency using SH-SY5Y neuroblastoma cells. Methods: Dopamine pathway proteins were identified with Western Blotting. Dopaminergic differentiation was attempted using all-trans retinoic acid (ATRA) with dopamine detection via HPLC-ECD post alumina extraction. Treatment with L-DOPA provided SH-SY5Y with excess precursor. RT-PCR was used to determine the expression of markers of mature neurons. Results: Western Blot screening identified AADC, dopamine β-hydroxylase and tyrosine hyrdoxylase proteins, indicative of a dopaminergic pathway. ATRA was unsuccessful in producing dopamine from the cells. L-DOPA treatment however, generated dopamine first visible as a HPLC-ECD peak 30 minutes post-incubation. Prior to this, SH-SY5Y dopamine synthesis from L-DOPA has never been documented. This de novo synthesis is then inhibited using benserazide to form our AADC deficiency cell model. RT-PCR showed that SH-SY5Y cells express markers of mature neurons in its 'native' state and is not affected by L-DOPA and benserazide treatment. This cell model will potentially benefit many areas of AADC deficiency research. Conclusion: SH-SY5Y cells produced HPLC-ECD measureable amounts of dopamine with the addition of L-DOPA. Our model of AADC deficiency is generated by quelling the dopamine production with Benserazide.

Keyword: AADC deficiency; Dopamine; SH-SY5Y; Retinoic acid; L-DOPA