[LBS.005] Hydroxyurea Restores the SMN2 Gene Expression in Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by the functional loss of the survival motor neuron gene (SMN). Over 95% of the SMA patients are missing the SMN1 gene but retain at least one copy of the homologous SMN2 gene. The SMN2 gene is transcribed, predominantly, into a truncated mRNA lacking exon 7. In this study, we seek to identify a therapeutic agent for SMA by attempting to restore the SMN2 gene function in human lymphocytes using histone deacetylator (HDAC) inhibitor hydroxyurea (HU). HU has been shown to increase the expression of human fetal hemoglobin gene in sickle cell anemia. Fifteen cultured lymphoblastoid cell lines derived from 12 SMA patients (5 type I, 4 type II, and 3 type III) and 3 control individuals were treated with HU in six concentrations (0, 500 ng/ml, 5mg/ml, 50 mg/ml, 500 mg/ml, and 5mg/ml) and over three time points (24h, 48h, 72h). Each dose and time point was paired with a vehicle-treated control from the same cell line. RT-PCR and quantitative densitometry were used to detect the changes of the SMN mRNA species. Western blot analysis was used to detect the changes in intact SMN protein. HU treatments resulted in a dose-related increase in the SMN full-length (fl) to truncated (tr) transcript ratio. At 48h of treatment, the fl/tr ratio increased significantly in all SMA cell lines. The ratios at 0 ng/ml, 500 ng/ml, 5 mg/ml, 5 0mg/ml, 500mg/ml, and 5 mg/ml HU treatments are: type I – 0.44, 0.35, 0.44, 0.81, 1.37** and 1.11*; type II – 0.42, 0.48, 0.68, 0.85**, 0.90** and 0.65; type III – 0.47, 0.34, 0.46, 0.79*, 0.94**, and 0.92**. Non-SMA cell lines did not show any significant change at any concentration of the HU treatments. HU treatments also resulted in a time-related increase in the fl/tr ratio of SMN transcripts in all SMA cell lines. Using a concentration of 50 mg/ml, the ratios at 24, 48 and 72 hours of treatment are: type I – 0.67, 0.99*, and 0.92*; type II – 0.91, 1.02**, and 1.19***; type III – 0.79, 0.82, 2.25. Vehicle-treated controls and non-SMA cell lines did not show significant changes at any time point. Western blot analysis showed an increase in SMN protein levels in 9 of 12 (75%) SMA cell lines and one of 2 control cell lines after the HU treatments. The concentration and time to reach peak responses varied from 500 ng/ml to 500 mg/ml and from 24 to 48 hours. Higher concentrations and longer treatment periods resulted in decrease in SMN protein levels in all cell lines when compared with the vehicle-treated paired controls. These results indicate that hydroxyurea treatment increases SMN2 gene expression in human SMA lymphoblastoid cell lines. This finding provides a strong biochemical basis for a clinical trial using hydroxyurea as a therapeutic agent for SMA. (*p=0.05, **p=0.01, ***p=0.001)

Muscular Dystrophy Association, U.S.A.; Spinal Muscular Atrophy Foundation

Dr. Grzeschik, Dr. Bartolotta, Dr. Daly, and Dr. Wang have nothing to disclose.

Hydroxyurea has not been approved for clinical treatment of SMA. The current study is conducted under IND #67.625.