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Beta-lactam antibiotic offers neuroprotection in a spinal muscular atrophy model by multiple mechanisms

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Background

Spinal muscular atrophy (SMA) is the most common genetic neurodegenerative disease leading to death in childhood.[1] SMA is characterized by the loss of spinal cord anterior horn neurons and progressive denervation of skeletal muscles. SMA is caused by deletion or mutation of the telomeric copy of human survival motor neuron gene 1 (hSMN1) and retention of the hSMN2 gene.[2,3] SMA animal models are extremely useful in studying the mechanism of SMA-related motoneuronal death, and may provide an *in vivo* system for testing a potential SMA therapy. In mice there is one survival motor neuron gene which is equivalent to SMN1. Complete loss of this gene results in embryonic lethality, due to the essential role of SMN in the assembly of small nuclear ribonucleoproteins (snRNPs).[4,5] SMN2 can produce the functional SMN protein required for all cells, including motoneurons. Expression of SMN lacking exon 7 (SMNΔ7) under the SMN promoter in severe SMA mice modulates the phenotype: on average, the mice live for two weeks.[6] Earlier works reported that, ceftriaxone, a β-lactam antibiotic slows the loss of muscle strength and extend survival in amyotrophic lateral sclerosis (ALS) in mice. Ceftriaxone prevents motoneuron loss in a dose-dependent manner. This effect may be linked due to improvement of glutamate clearance in spinal cord explants as expression of excitatory amino acid transporter-2 is increased. Indeed, ceftriaxone is neuroprotective *in vitro.*[7] Based on the above observation Nizzardo *et al* proposed this study to test the therapeutic effect of ceftriaxone in SMA.

Study Design

To study of effect of β-lactam antibiotic as neuroprotectant and the possible mechanism, in spinal muscular atrophy, the authors used a triple mutant SMA mouse. This animal model was genotyped using PCR based assay and carries two transgenic alleles and a single targeted mutant. Ceftriaxone was given via intraperitoneal injection against suitable control and analysis was done daily for clinical signs of the disease. Righting reflex and Hind limb suspension test were performed to analyze improvement for neuromuscular function of diseased mice. Histological analysis of muscles of hind limbs for total tibialis anterior (TA), cross sectional area, total TA myofiber number and myofiber diameter was performed by the author. Spinal cord sections were subjected to immunohistochemistry using monoclonal antibodies against motoneuron specific markers SMI 32 antigen followed by secondary antibody AlexFlour 488. Purified RNA isolated from spinal cord followed by cRNA preparation and oligonucleotide microarray hybridization. Biotinylated cRNA was hybridized to Affymetrix GeneChip Mouse Genome 430A 2.0 array at 47°C overnight and visualized on GeneArray 2500 Scanner. RNA from four groups of selected animals was reverse transcribed on Real time PCR system. Spinal cord protein was extracted from experimental animals and subjected to semi quantitative western blot. The membranes were probed with mouse anti SMN, anti EAAT2 antibody, rabbit anti BAX, anti PRG2, anti TDP 43, anti FUS and anti actin antibodies followed by use of secondary antibodies and chemiluminescence detection techniques. The staining sections and proteins density were measured with NIH Image software. Kaplan Meier analysis was used on lifespan using log rank post hoc test. ANOVA and Tukey-post hoc analysis of growth curve was performed. Student's test was used for statistical analysis and upper significance level of 0.05 was used.

Implications

This study showed the neuroprotective effect of ceftriaxone on motoneurons and motor unit integrity by modification of gene expressions. The treatment of SMA mice with ceftriaxone increased survival time by 31.6%, slightly changed SMN defect mediated pathology and ameliorated motor dysfunction. A number of unregulated genes containing one or more NF-κB binding sites were observed after treatment. The author reported both GLT1 mediated reduction of excitotoxicity and activation of NRF2 related factors which may contribute to phenotypic amelioration. Out of several genes involved in transcription and RNA processing few of them including FUS, plastin 3 got down-regulated in SMN. These can be used as biomarkers to correlate with disease progression, however rel-

evance of these genes to disease in humans and efficacy of other drugs need to be studied.

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