Target Intestinal Microbiota to Alleviate Disease Progression in Amyotrophic Lateral Sclerosis

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ABSTRACT

Purpose: Emerging evidence has demonstrated that gut microbiome plays essential roles in the pathogenesis of human diseases in distal organs. Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive loss of motor neurons. Treatment with the only drug approved by the US Food and Drug Administration for use in ALS, riluzole, extends a patient's life span by only a few months. Thus, there is an urgent need to develop novel interventions that for alleviate disease progression and improve quality of life in patients with ALS. Here we present evidence that intestinal dysfunction and dysbiosis may actively contribute to ALS pathophysiology.

Methods: We used G93A transgenic mice as a model of human ALS. The G93A mice show abnormal intestinal microbiome and damaged tight junctions before ALS disease onset. The mice were given 2% butyrate, a natural bacterial product, in the drinking water.

Results: In mice fed with butyrate, intestinal microbial homeostasis was restored, gut integrity was improved, and life span was prolonged compared with those in control mice. At the cellular level, abnormal Paneth cells—specialized intestinal epithelial cells that regulate the host–bacterial interactions—were significantly decreased in the ALS mice treated with butyrate. In both ALS mice and intestinal epithelial cells cultured from humans, butyrate treatment was associated with decreased aggregation of the G93A superoxide dismutase 1 mutated protein.

Implications: The findings from this study highlight the complex role of the gut microbiome and intestinal epithelium in the progression of ALS and present butyrate as a potential therapeutic reagent for restoring ALS-related dysbiosis. (*Clin Ther*. 2017;39:322–336)

Key words: ALS, butyrate, dysbiosis, intestinal permeability, microbiome, tight junctions.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Most patients with ALS die 3 to 5 years after diagnosis, due to respiratory paralysis.[1] The lifetime risk for ALS is ~1 in 472 in women and 1 in 350 in men in worldwide. Because ALS is an age-dependent disease, as the US population increases and ages, an increase in the prevalence of ALS can be anticipated. Currently there is no intervention that can significantly change the course of the disease.[2] Treatment with the only drug approved by the US Food and Drug Administration for use in ALS, riluzole, extends a patient's life span by only a few months. Thus, there is an urgent need to develop novel interventions that slow disease progression and improve quality of life in patients with ALS.

Many cases of familial ALS (20%–25% percentage of familial ALS cases) are associated with mutations in the Cu/Zn superoxide dismutase gene (*SOD1*). ALS-model mice, G93A, harbor human ALS–causing *Sod1* mutations that recapitulate the neuron and muscle impairment in patients with ALS. These mice are extensively used to investigate the pathomechanisms of ALS and trial therapeutics.[3–5] Our recent study revealed an exciting phenotype that linked aberrant microbial and intestinal homeostasis to disease progression in ALS mice.[6] G93A mice have dysbiosis (an imbalanced gut-bacterial profile), disrupted tight

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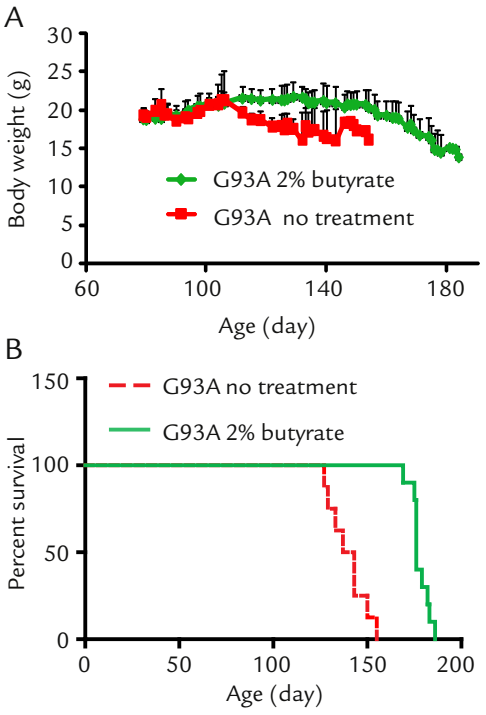

Figure 1. Effects of sodium butyrate on weight and survival in G93A amyotrophic lateral sclerosis–model mice with treatment (2% sodium butyrate from age 63 days, for 2.5 months; n = 10) or without treatment (control; n = 8). Sodium butyrate was associated with weight loss prevention (A) and extended survival times (B). The treated mice died on days 169, 175, 176 (n = 4), 179, 182, 183, and 186. The control mice died on days 127, 129, 133, 137, 143 (n = 2), 150, and 155.

junction (TJ) structure, and increased intestinal permeability (leaky gut). These changes occur in young G93A mice before ALS symptom onset, suggesting that impaired gut–neuromuscular crosstalk may actively contribute to ALS progression and pathogenesis.

The intestinal microbiome community modulates numerous aspects of human physiology and is a crucial factor in the development of chronic diseases.[6] Intestinal epithelial cells are consistently exposed to bacteria, a process that plays a key role in development, renewal, and immunity.[7–9] Frequent microbial challenges to epithelial cells trigger discrete signaling pathways, promoting molecular changes, such as the secretion of cytokines and chemokines, and alterations to molecules at the epithelial cell surface. A lack of intestinal homeostasis and an aberrant microbiome play essential roles in neurologic diseases, such as autism and Parkinson's disease.[3,4] However, restoring intestinal homeostasis and gut microbiome in ALS is unexplored.

In the current study, we hypothesize that restoring microbiome and intestinal homeostasis delays disease onset and progression in ALS. A leaky gut can contribute to the altered microbiome environment, which leads to a reduced production of beneficial bacterial products. Indeed, our previous study demonstrated that G93A mice show an abnormal microbiome profile with a reduced population of butyrate-producing bacteria. Remarkably, after being fed with the natural bacteria product butyrate, the G93A mice exhibited a delay in the onset of ALS symptoms and a prolonged life span. The present study explored butyrate for restoring intestinal homeostasis and microbiome, thus opening a new avenue in targeting the gut microbiota–butyrate axis for ALS treatment.

MATERIALS AND METHODS

Animals

G93A[5] and age-matched wild-type (WT) mice were used in this study. All experiments were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of Rush University and the University of Illinois at Chicago Committee on Animal Resources.

Butyrate Treatment in G93A Mice

G93A mice aged 8 to 9 weeks weeks were randomly assigned to 1 of 2 groups. The butyrate-treated group received sodium butyrate (catalog number 303410; Sigma-Aldrich, St. Louis, Missouri) at a 2% concentration in filtered drinking water. Butyrate treatment starts at age 63 days, and finishes after 2.5 months. The control group received filtered drinking water without sodium butyrate. All mice were provided with water ad libitum and maintained in a 12-hour dark/light cycle. All animals were weighed and received a detailed clinical examination, which included assessments of appearance, movement and behavior patterns, skin and hair conditions, eyes and mucous membranes, respiration and excreta.