Efficacy and Safety of Bumetanide in Patients with Amyotrophic Lateral Sclerosis: A Randomized Controlled Clinical Trial

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Abstract

Background: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease

characterized by the terminal degenerative disease of the motor units. This clinical trial was aimed to investigate the modulatory effect of bumetanide on physiological symptoms of ALS patients.

Methods: This was a double-blind, placebo-controlled trial in which ALS patients were randomized 1:1 to receive bumetanide (2 mg daily) or matching placebo group for up to 4 months. Motor Unit Number Index (MUNIX), motor unit size index (MUSIX), and ALS Functional Rating Scale, revised (ALSFRS-R) was assessed before and after treatment as following bumetanide treatment.

Results: 18 patients were allocated to bumetanide and 18 to the placebo group. In final analysis, 16 patients in bumetanide and 15 patients in the placebo group completed the trial. Patients in the placebo group showed a significant decrease in MUNIX value for all examined muscles after treatment, while MUNIX value for trapezius in bumetanide group increased significantly ($p < 0.05$). MUZIX value for tibialis anterior and trapezius ($p < 0.05$) improved significantly after bumetanide administration, whereas trapezius ($p < 0.05$) and abductor pollicis brevis ($p < 0.01$) in the placebo group showed a significantly decreased value. ALSFRS-R score decreased significantly in the placebo group after treatment ($p < 0.001$), but ALSFRS-R in bumetanide group improved significantly ($p < 0.05$). Three adverse effects (polyuria, vertigo, orthostatic hypotension) in bumetanide group were judged to be related to bumetanide.

Conclusion: Bumetanide treatment might be effective in modulation of ALS symptoms possibly due to the hyperpolarization of GABA actions and mitigation of cortical hyperexcitability.

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by the terminal degenerative disease of the motor neurons (MNs) in the cortex, brain stem, and spinal cord [1, 2]. MNs degeneration leads to progressive muscle weakness, muscle atrophy, fasciculations, spasticity, and paralysis which ultimately result in death usually within 3–5 years after the onset of disease [2, 3]. Moreover, up to 50% of patients are involved by the non-MNs degeneration characterized by the detectable cognitive and behavioral impairment in ALS patients. While 10% of all ALS cases are considered to be familial, the remaining 90% of patients suffer from sporadic form 4. From the pathological point of view, mutations in superoxide dismutase 1 (SOD1) gene are always found both in familial and sporadic forms of ALS lead to the increase of neurotoxicity because of protein misfolding [5].

The Motor Unit Number Index (MUNIX) is a promising electrophysiological method to estimate the number of surviving motor units in ALS patients. It has been demonstrated that MUNIX value declines over time in ALS patients [6]. MUNIX is easy to perform and needs a minimal number of stimuli which is well tolerated by the ALS patients [7]. Besides, the progression of ALS can be monitored with the ALS

Functional Rating Scale, revised (ALSFR-S) which also includes respiratory function. Clinical assessment of disease provides the functional outcomes as a combination of central parameters, motor neuron degeneration, and compensatory sprouting 8.

Cortical hyperexcitability has been recognized as a key pathogenic mechanism in ALS, promoting the development of lower motor neuron degeneration [9]. Also, common pathways of neurodegeneration in ALS seem to be linked to the excitotoxicity as chronic dysregulation of the intracellular calcium homeostasis that could be affected by dysregulation of inhibitory and excitatory synaptic neurotransmission [10]. Gamma-aminobutyric acid A (GABAA) is the most widely distributed inhibitory neurotransmitter in the adult central nervous system (CNS) and its receptors are responsible for the fast-inhibitory synaptic transmission in the CNS [11]. Recent investigations have shown an up-regulation of the β1 and a down-regulation of the α1 subunit in the primary motor cortex of ALS patients [12]. Moreover, dysfunction of GABAergic intracortical inhibitory circuits has been found in ALS, reflecting by the decline in short-interval intracortical inhibition (SICI) [13].

Bumetanide, a specific Na-K-2Cl cotransporter (NKCC1) antagonist, has shown that reduces intracellular chloride [Cl)]i and switches GABA from excitation to inhibition [14]. It has been documented that bumetanide demonstrates positive effects on both physical and behavioral aspects of CNS disorders such as epilepsy [15], schizophrenia [16], autism spectrum disorder (ASD) [17], and Parkinson disease (PD) [18]. Hence, this clinical trial was designed to investigate the possible modulatory effects of bumetanide administration on related physiological symptoms in ALS patients.

Methods

Trial design and patients

This randomized, double-blind, placebo-controlled study was conducted in Firoozgar hospital in Iran University of Medical Sciences. We recruited eligible patients from the outpatient clinic and participants were considered eligible if they were diagnosed with ALS according to the El-Escorial diagnostic criteria for ALS. Besides, eligible patients were aged 18 to 80 years, able to swallow tablets/capsules, and showed slow vital capacity (SVC) greater than or equal to 50% predicted for sex, age and height. We excluded participants if they had kidney or liver dysfunction, history of laryngeal dystonia and/or akathisia, history of moderate or severe brain injury or stroke, complicated diabetes mellitus, and history of cardiac arrhythmia.

Ethical Standards

All procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The protocol of this study was reviewed and approved by the Institutional Review Board of the Iran University of Medical Sciences (IR.IUMS.REC.1399.904) and was registered in the Iranian Registry of Clinical Trials (IRCT20201020049089N1) on 15/12/2020.

Randomization

Participants were randomly assigned in a 1:1 ratio to groups of bumetanide or placebo by using a block randomization scheme. The sample size was calculated based on a previous study by Lemonnie *et al* reporting that bumetanide 2 mg significantly improves clinical global impressions (CGI) score in 38.5% of patients with ASD [17]. Accordingly, with the 95 % confidence level, power set at 80%, and a potential attrition rate of 10%, the calculated sample size was 18 for each group of trial. All participants and their caregivers, clinicians, researcher and data analysts were blinded to treatment assignments until final data analysis.

Treatment

This study consisted of two phases including treatment phase (4 months) and follow up phase (1 month). Choosing of bumetanide dose was based on our prior investigations, reporting the effectiveness of bumetanide 2 mg on the reduction of seizure frequency in patients with epilepsy [15]. Hence, bumetanide 2 mg tablet (Almus Pharmaceuticals, UK) was administrated daily in bumetanide group and patients in the placebo group revived daily placebo tablet which was identical in size, appearance and taste with bumetanide. Bumetanide side effects were monitored through monthly blood serum analysis during the study. All patients were visited weekly to monitor their physiological symptoms.

MUNIX procedure

MUNIX analysis was developed by Nandedkar for assessment of number and size of motor units in patients with motor neuron disease [19]. The primary endpoint measurement in this study was the MUNIX index. We performed the MUNIX in ALS patients before and after treatment in muscles from lower and upper extremities, including tibialis anterior (TA), abductor pollicis brevis (APB), abductor digiti minimi (ADM), and trapezius. Motor unit size index (MUSIX) as an indicator of the motor unit surface was also calculated by EDX machine. Participants were positioned appropriately and tests were performed by the same neurologist through a standard electromyography instrument.

ALSFRS-R

The secondary endpoint measurement was ALSFRS-R score which is a well-established and widely distributed score for assessment of physical function in the domains of gross and fine motor function, bulbar symptoms, and respiratory function in ALS patients [20, 21]. ALSFRS-R is based on 12 items, each of which is rated on a 0-4 point scale. The score of total functional disability thus ranges from 0 (maximum disability) to 48 (normal) points [22]. In this study, we used the ALSFRS-R instrument before and after treatment in both groups of trial.

Statistical Analysis

Data were analyzed using the GraphPad Prism 8.1.1 (GraphPad, San Diego, CA, USA) statistical software package. Continuous values were expressed as mean ± standard deviation (SD) and analyzed using the

Student t-test. The Pearson chi-square test was used to examine the categorical data. In all analyses, the significance level was set at $p < 0.05$.

Results

Characterization of patients

From February 2019 to February 2020, a total of 45 patients were screened for the study. Of this, 9 patients were excluded because they did not meet our inclusion criteria. Therefore, 36 cases were randomized and divided into groups of bumetanide (n=18) and placebo (n=18). Three patients in the placebo and two patients in the bumetanide group were unwilling to continue the study and excluded from the final analysis (Figure. 1). The baseline demographics and clinical data of remained participants are presented in Table 1. The patients ranged in age from 22 to 75 years and the mean age of them was 54.21±14.7. years. Fifteen patients (48.4%) were female and sixteen patients (51.6) were male. There were no significant differences between the two groups of study regarding patient variables. Also, we observed no mortality events or series complications among the participants during the trial.

Primary endpoint measurement

Figure 2 presents the comparison of mean MUNIX value for APB (2A), ADM (2B), TA (2C), and trapezius muscle (2D) in bumetanide and placebo groups before and after treatment. The mean value of MUNIX for all examined muscles showed a significant decrease compared to the baseline ($p<0.05$) in the placebo group. Although MUNIX values in bumetanide group increased slightly for all examined muscles, this increase for trapezius muscle was significant when compared to the baseline (p0.05). As shown in Figure 3, MUSIX value for TA (3C) and trapezius muscle (3D) in bumetanide group improved significantly ($p<0.05$), while this value for APB (3A) and trapezius muscle (3D) showed a significant decrease ($p<0.05$).

Secondary endpoint measurement

As depicted in Figure 4, ALSFRS-R score significantly improved in bumetanide group after treatment with bumetanide in comparison with the baseline (27.7±2.2 vs 29.3±2.4, $p=0.032$). In contrast, ALSFRS-R score for the placebo group showed a significant decrease after treatment when compared to the baseline (38.2±2.1 vs 35±2.3, $p=0.001$).

Adverse effects

The most common adverse effect observed in bumetanide group was polyuria in 13 patients (81%). Other reported adverse effects by patients were vertigo in 6 patients (37.5%) and orthostatic hypotension in 4 patients (25%) that occurred in the first week of the treatment phase and were resolved well after one week.

Discussion

Our results from this clinical trial showed potential benefits of bumetanide 2 mg in improving primary and secondary endpoints of the study, suggesting that bumetanide could be effective in modulating ALS physiological symptoms. Bumetanide was safe and well-tolerated at a dose of 2 mg and could improve ALSFRS-R score, MUNIX and MUSIX values in examined muscles.

While the concentration of the bumetanide remains low in the brain after systemic administration [23], recent investigations indicated that bumetanide can control some aspects of neurological and neuropsychological disorders [24]. Prior studies have demonstrated that bumetanide improves ASD behaviours as assessed by the Childhood Autism Rating Scale (CARS) and CGI in children [17]. Also, it has been shown that bumetanide restores altered EEGs power spectra in an adolescent with ASD [25]. Cortical hyperexcitability has been found as a key pathophysiologic process in ALS. However, the exact mechanisms underlying the development of hyperexcitability at the cortical level are not fully understood [13, 26]. Recent studies have reported degeneration of inhibitory cortical interneurons, proposing that disinhibition of cortical interneurons has a substantial contribution to the development of cortical hyperexcitability in ALS [27]. This report has been supported by the findings of reduced expression of the GABAA receptors in ALS motor cortices and the ubiquitous loss of binding of the GABAA receptor ligand [11C] flumazenil in ALS patients. Besides, a neuroimaging study by PET showed that binding of the benzodiazepine antagonist flumazenil was declined in the motor cortex and several extramotor brain regions of patients with ALS [12, 28]. Furthermore, a remarkable loss of cortical inhibition or corticomotor hyperexcitability in ALS patients was reported by transcranial magnetic stimulation (TMS) investigations [29-31].

From the cellular point of view, the efficacy of GABAergic inhibition is strongly correlated with the levels of intracellular chloride concentration ([Cl−]i) [32]. Increased levels shift the polarity of GABA actions from hyperpolarizing to depolarizing and even excitatory. This phenomenon has been reported in a broad range of neurological disease including epilepsies, chronic pain, traumatic injuries, and spinal cord lesions [33]. It has been reported that bumetanide restores physiological levels of [Cl−]i and hyperpolarizing GABA actions, mitigating the severity of the symptoms in several neurological disorders such as PD, ASD, and epilepsies [15, 17, 18, 34]. Interestingly, a recent report has shown downregulation of Na–K–Cl cotransporter 1(NKCC1) protein following bumetanide treatment that may be responsible for its antiepileptic effects [35]. Also, another investigation indicated that bumetanide is efficient on the reduction of seizure frequency in adult patients with temporal lobe epilepsy (TLE) through the microstructural reorganization that mostly affects the epileptic regions [15, 24]. Even though bumetanide concentration in the brain environment remains low after administration, investigations on Huntington and Down's model indicated that restoring the inhibitory function of GABA by bumetanide might contribute in memory improvement via inducing modifications in synaptic plasticity patterns [36, 37].

Limitations

There are several limitations to the present study. A major limitation of our study is the small sample size of the population and the short duration of the study. Therefore, more extensive randomized clinical trials with larger sample sizes are required to confirm our findings. Another limitation is the absence of sensitive and specific biomarkers of ALS to monitor and evaluate patient outcomes. ALS biomarkers have the potential to help clinicians and investigators better understand the disease, enhance the design of clinical trials, develop novel therapeutics, and improve patient results.

Conclusion

To our knowledge, this is the first study to investigate the effectiveness of bumetanide on the modulation of physiological symptoms in ALS patients. Our findings showed that bumetanide 2mg treatment may lead to the modulation of ALS symptoms possibly due to the hyperpolarization of GABA actions and mitigation of cortical hyperexcitability. If the efficacy of bumetanide is proven in large-scale investigations, it will be used as a supplement therapy to control symptoms in ALS patients.

Declarations

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Conflict of interest: The authors have no conflicts of interest that are directly relevant to the content of this article.

Data availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent to participate: Written informed consent was obtained from all individual participants included in the study prior to starting any study-related procedure.

Consent for Publication: Not applicable.

Code availability: Not applicable.

Author Contributions

SM, BHA, and MTJ contributed to the study conception and design. SM and MS carried out the statistical analysis of the study. BHA, MA, SAH, ES, and MP contributed to the study management and data collection. BHA and MTJ supervised operational aspects of the trial and data collection. SM, BHA, and MTJ reviewed and commented on the study results. The first draft of the manuscript was written by MS and SM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Table

Table 1: Characteristics of the patients in two groups of study.

Variables	Bumetanide (n=16)	Placebo (n=15)	p value
Age (years) [a]	56.10±12.06	52.11±17.74	0.467
Gender (n, %) [b]			
Male	10 (62.5%)	6 (40%)	0.886
Female	6 (37.5%)	9 (60%)	
Type of disease (n, %) [b]			
Sporadic	15 (93.8%)	14 (93.3%)	0.962
Familial	1 (6.2%)	1 (6.7%)	
Onset site (n, %) [b]			
Spinal	9 (56.3%)	11 (73.3%)	0.320
Bulbar	7 (43.8%)	4 (26.7%)	
Duration of disease (months) [a]	22.01±9.6	20.06±12.5	0.628
Riluzole consumption (n, %) [b]	11 (68.8%)	10 (66.7%)	0.901

[a] Continues data are presented as mean ± standard deviation.

[b] Categorical data are presented as frequency (Percentage).

Figures

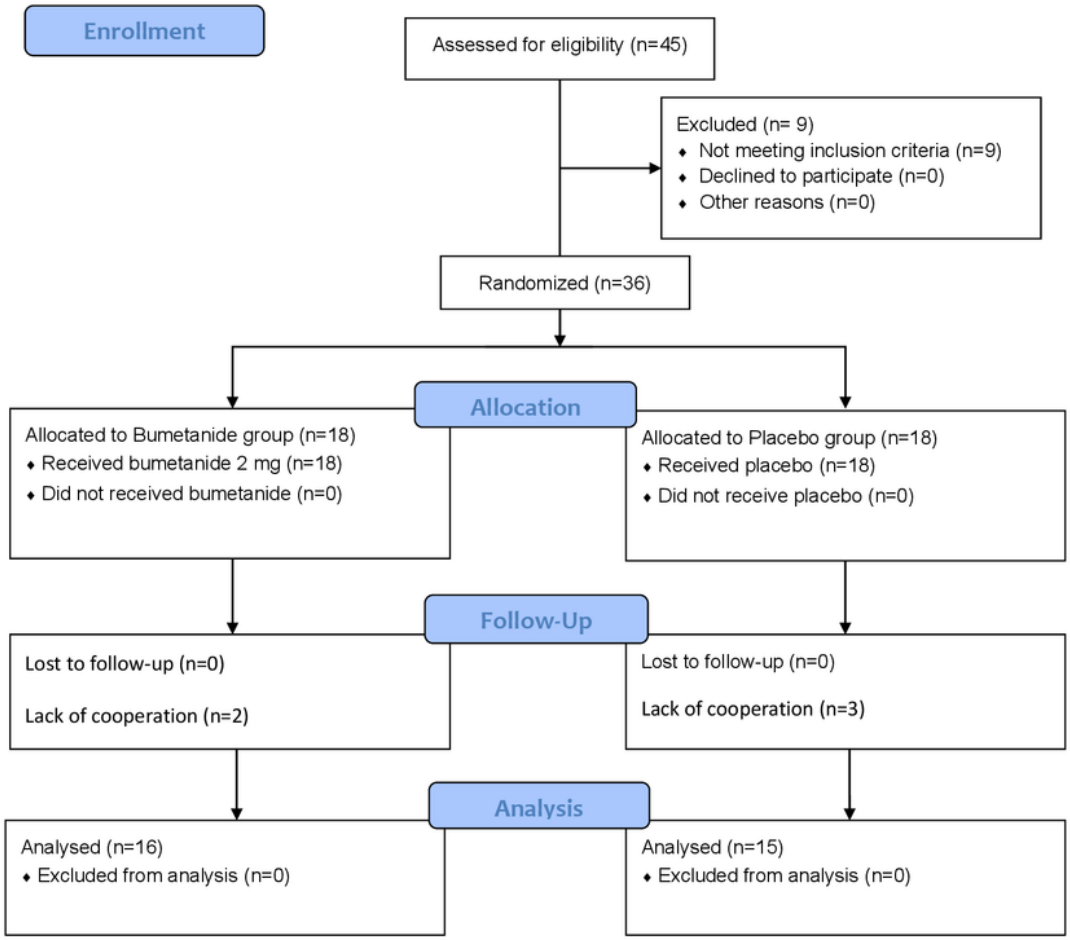

Figure 1

Schematic diagram of the protocol used. A total of 45 ALS patients were recruited for the trial. 9 patients were excluded for various reasons and the remaining 36 were allocated into bumetanide and placebo groups. 5 cases were withdrawn from the study due to lack of cooperation.

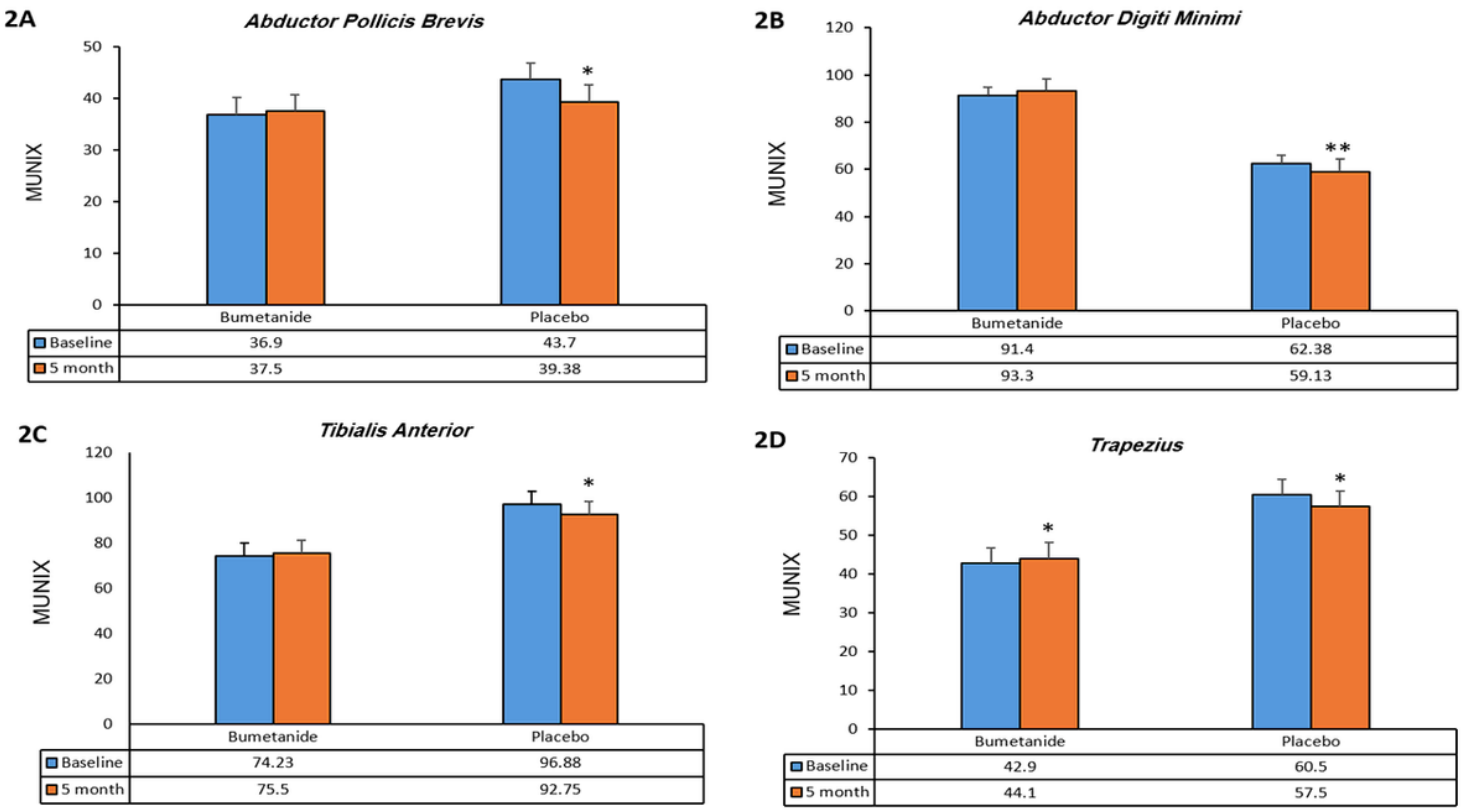

Figure 2

Comparison of MUNIX value for abductor pollicis brevis (2A), abductor digiti minimi (2B), tibialis anterior (2C), and trapezius (2D) in bumetanide and placebo groups before and after treatment (* p 0.05, ** p 0.01). MUNIX: Motor Unit Number Index.

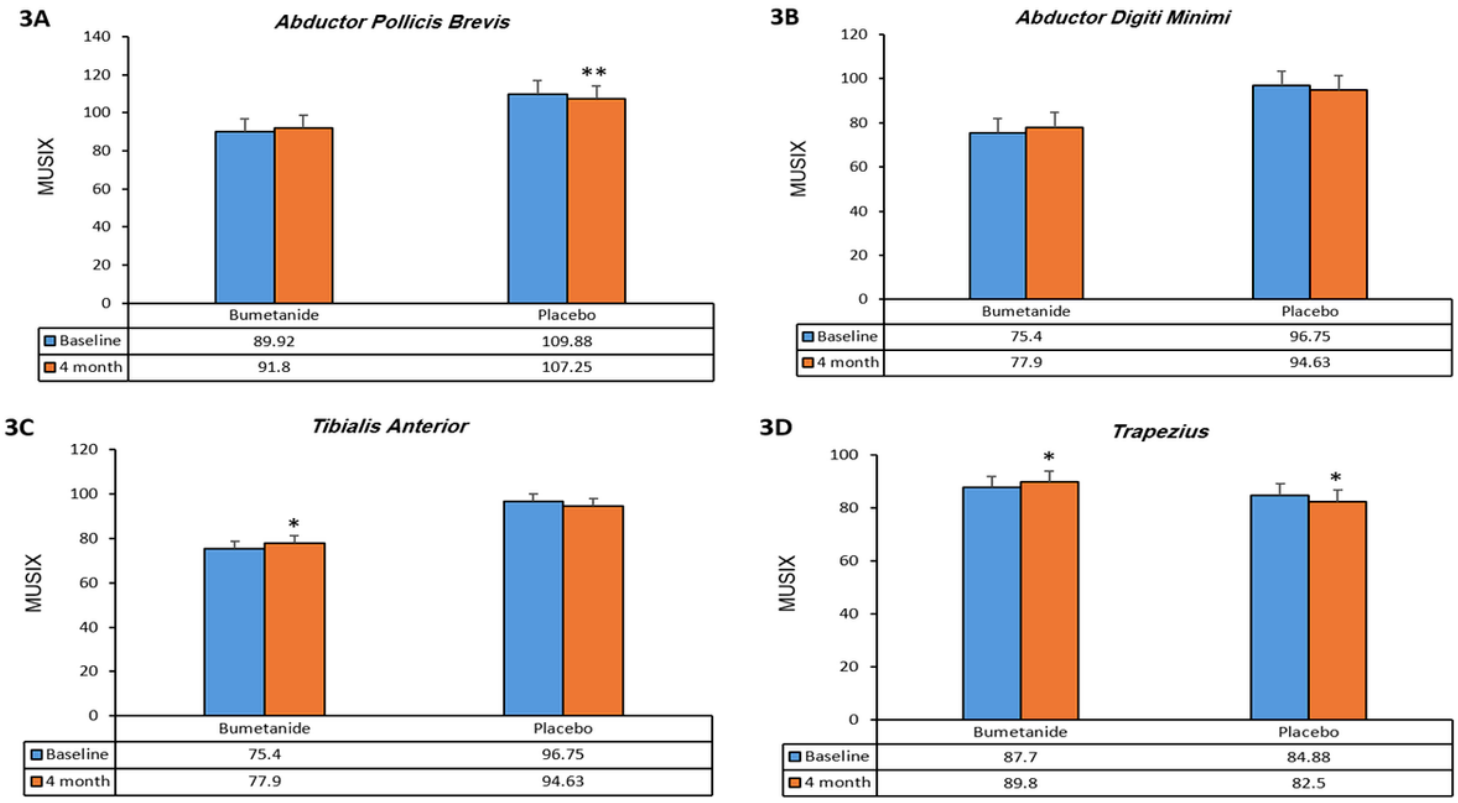

Figure 3

Comparison of MUSIX value for abductor pollicis brevis (3A), abductor digiti minimi (3B), tibialis anterior (3C), and trapezius (3D) in bumetanide and placebo groups before and after treatment (* p 0.05, ** p 0.01). MUSIX: Motor Unit Size Index.

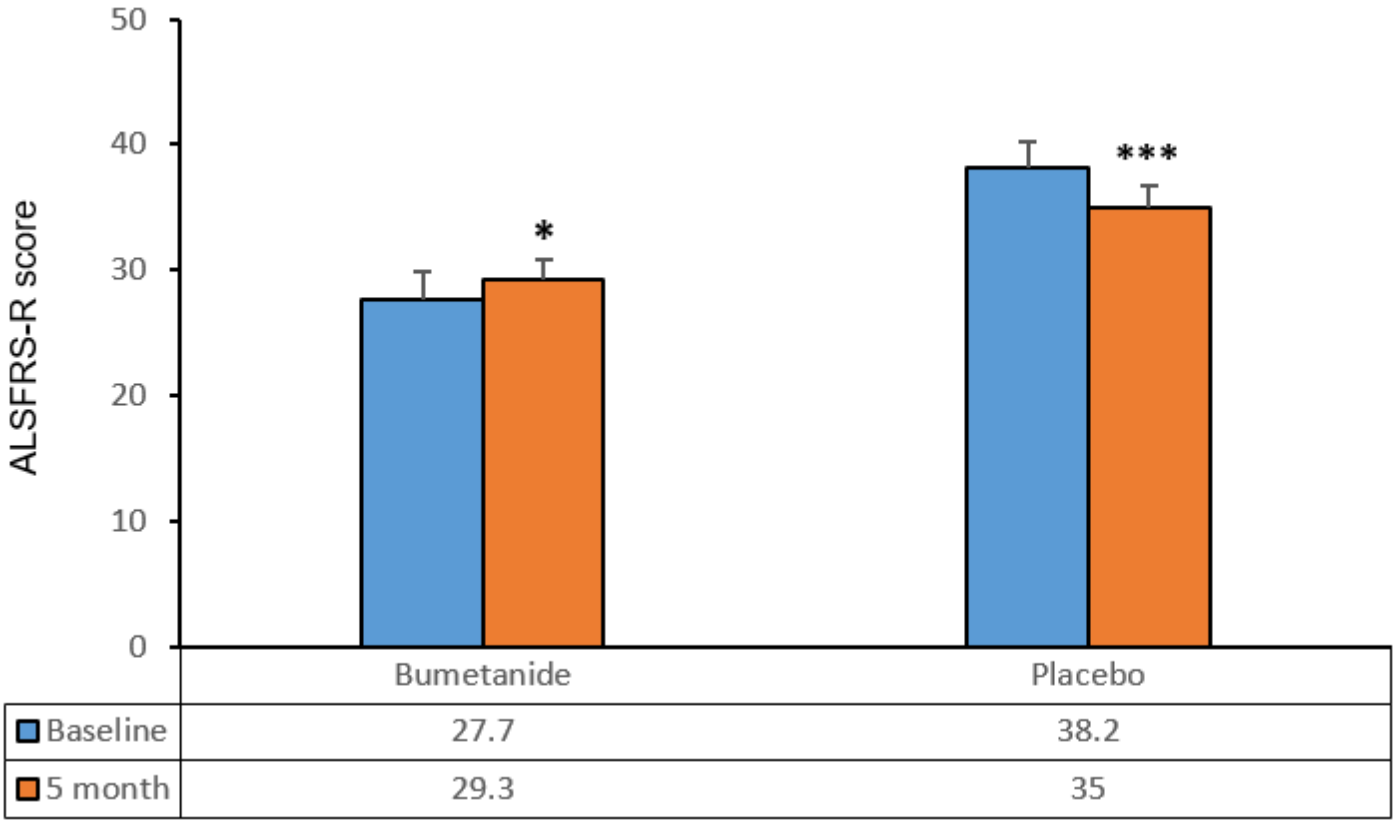

Figure 4

Comparison of ALSFRS-R score in bumetanide and placebo groups before and after treatment (* p 0.05, *** p 0.001). ALSFRS-R: ALS Functional Rating Scale, Revised.