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TOLERABILITY OF LITHIUM CARBONATE ASSOCIATED TO RILUZOLE IN AMYOTROPHIC LATERAL SCLEROSIS

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ABSTRACT

Objective: to describe the adverse events and tolerability of lithium carbonate plus riluzole used in a trial to reduce the progression of amyotrophic lateral sclerosis. Methods: An 18-month, randomized controlled clinical trial, phase 2 open parallel, with two arms was used. The experimental group (n=30) received riluzole 100 mg/day plus lithium carbonate (blood level: 0.4 to 0.8 mEq/l), and the control group (n=30) received riluzole 100 mg/day. The participants were evaluated each 3-4 months. The primary outcome was time of tracheostomy or death. The secondary outcomes were ALSFRS scale, muscle strength, forced vital capacity, hemoglobin saturation and neurophysiological biomarkers. Results: Twenty-two patients who used lithium carbonate and riluzole discontinued the medication. Of these, eight (26.7%) considered the drug ineffective and 14 (46.7%) experienced adverse events. The trial was halted prematurely for adverse events. One patient had respiratory distress with full recovery with withdrawal. In an intention to treat analysis, the survival functions did not differ significantly between groups (Kaplan-Meier, log-rank 0.38). In a *per protocol* analysis,the survival function was better in comparison to the control group, with a mean survival of 14.72 months [95% CI 13.19-16.25]), whereas, in the experimental group, the mean survival was 10.59 months [95% CI 7.12-14.07] (log-rank 0.04). For the secondary outcomes, there were no differences between the groups. Conclusions: There was low compliance, lack of effectiveness, a high incidence

of adverse events and occurrence of respiratory distress with lithium carbonate in amyotrophic lateral sclerosis. Trial registration number: RBR-2n5mtq; date of trial registration: 2015-07-28.

KEYWORDS: amyotrophic lateral sclerosis, motor neuron disease, lithium, survival, CMAP, motor unit, treatment, drug.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease that is characterized by the progressive loss of motor neurons in the brain, brain stem and spinal cord, thereby resulting in generalized weakness, muscle atrophy, paralysis, and eventual mortality within 3-5 years of disease onset.[1] Riluzole improves survival in patients, but its effect is only moderate.[2]

Treatment with either lithium alone or in conjunction with other antioxidant drugs showed improved motor function and reduced disease progression in a mouse model of ALS.[3] In addition, one preclinical study combined lithium and valproate and produced a greater and more consistent effect than monotreatment with either drug in delaying the onset of disease symptoms, decreasing neurological deficit scores, and prolonging life span.[4]

Furthermore, over the past five years, three large randomized clinical trials (5–7) showed low incidence of adverse events and no benefit with association of lithium and riluzole. Despite these negative trials, some authors have raised methodological questions in clinical studies that assess lithium efficacy in reducing ALS progression.[8]

Furthermore, there is a consensus that in ALS, clinical trials have long been limited by problems in choosing reliable endpoints or biomarkers.[9] In this context, neurophysiological biomarkers provide a novel quantitative electrophysiological technique that provides indirect measures of the number of functional lower motor neurons in a muscle. It has been suggested that these techniques may serve as a marker of the progressive loss of motor units, which is useful in ALS outcome.[10,11]

The aim of this study is to describe the adverse events and tolerability of lithium in this group of patients. Additionally, the authors try to clarify the concerns about the role of lithium carbonate as a neuroprotector in reducing the progression of the disease. We sought to

correlate laboratory biomarkers to clinical parameters to facilitate the assessment of outcomes in patients.

METHODS

A randomized controlled clinical trial, phase 2 open parallel, with two arms, was performed at the Reference Center for Neuromuscular Diseases of the State Secretariat of Health of the Federal District, from March 2014 to September 2015. The trial was not blinded because there was only one medical doctor prescribing and evaluating the patients.

Approval was obtained from the local Research Ethics Committee - FEPECS (Fundacao de Ensino e Pesquisa em Ciencias da Saude), 525 241/2014 Protocol. The clinical trial was registered under Rebec RBR-2n5mtq protocol.

Inclusion criteria were age above 18 years; a possible, probable or definite diagnosis of ALS according to the El Escorial Criteria[12]; exclusion of other diseases through skull and spinal cord magnetic resonance, laboratory tests and electromyography; forced vital capacity (FVC) above 50%. Exclusion criteria were FVC below 50% and the diagnosis of other diseases with similar clinical picture.

The sample calculation, after a retrospective study in the same center[13] estimated a total of 80 patients (40 in each arm) to have an 80% power to detect a 30% absolute reduction (from 60% to 30%) in the primary endpoint in the group treated with lithium, with a 5% two-sided type 1 error. The allocation was randomized using the iRandomizer® application and each participant signed a written consent form. The recruitment was conducted from March 2014 to March 2015.

Participants were randomly assigned in two groups: the first group (experimental group) with 30 patients receiving riluzole100 mg / day associated with lithium carbonate orally in scaled dose according to blood levels between 0.4 to 0.8 mEq/l[5-7] and the second (control group) with 30 patients receiving riluzole 100 mg / day isolated. The participants were evaluated each 3-4 months, with physical examination, laboratory blood tests, breathing tests (i.e., spirometry and oxyhemoglobin saturation observed in supine position) and surface electromyography.

The primary outcome was time for tracheostomy or death within 18 months or by observing the development of respiratory failure, with oxyhemoglobin saturation below 90% and

decrease in PaO_2 below 60 mmHg. It was discarded Chronic Obstructive Airway Disease by spirometry analysis.

The secondary outcomes were median and ulnaris right nerves CMAP (Compound Muscular Action Potential) area and amplitude[10] in right median and ulnar nerves in every three months; ALSFRS scores-Amyotrophic Lateral Sclerosis Functional Rating Scale[15] with a total score of 40 points; muscle strength - MRC - Medical Research Council[16], with a total score of 70 points; Forced Vital Capacity (FVC) in supine position and oximetry in the supine position for 15 minutes.

Every six months, it was made an interim analysis, which resulted in the trial interruption at 18th month, because there was in a serious adverse event.

The protocol for CMAP amplitude and area measurements, model and computation follow the model that was postulated by Nandedkar et al.[14] A self-adhesive disposal surface ground and two disc recording electrodes with 15 mm diameter were used. Measurements were performed using commercial Keypoint-Classic-electromyograph in the right *Adbutor Pollicis Brevis* (APB) and *Abdutor Digiti Minimus* (ADM).

Statistical analysis

Data were allocated in Office Excel 2010 charts and analyzed using SPSS (Statistical Package for Social Sciences), version 19.0. The confidence intervals were calculated assuming a Poisson distribution. Categorical variables were analyzed using chi-square test with the two-tailed Z test and Fisher's Exact Test. Quantitative variables were analyzed by the Student T test and Kruskal-Wallis Non-parametric test. The significance level was 0.05.

The survival functions for the patients were estimated by Kaplan-Meier method and compared using the log-rank test, with p significance level of 0.05.

RESULTS

Between March 2014 and June 2015, 65 patients were screened, 60 of whom were recruited from the Neuromuscular Reference Center (CRDN) and were randomly assigned to the lithium carbonate plus riluzole (experimental) group (n= 30) or to the riluzole alone (control) group (n=30). At 18 months, it was made an interim analysis and the study stopped for futility, because there was low compliance to lithium and a serious adverse event. Forty-four patients (74%) took at least 40% of the prescribed drugs. Compliance was better in the

riluzole alone group (93%) than in the lithium plus riluzole group (53.3%). During the study, no patient withdrew consent. Figure 1 shows the trial profile.

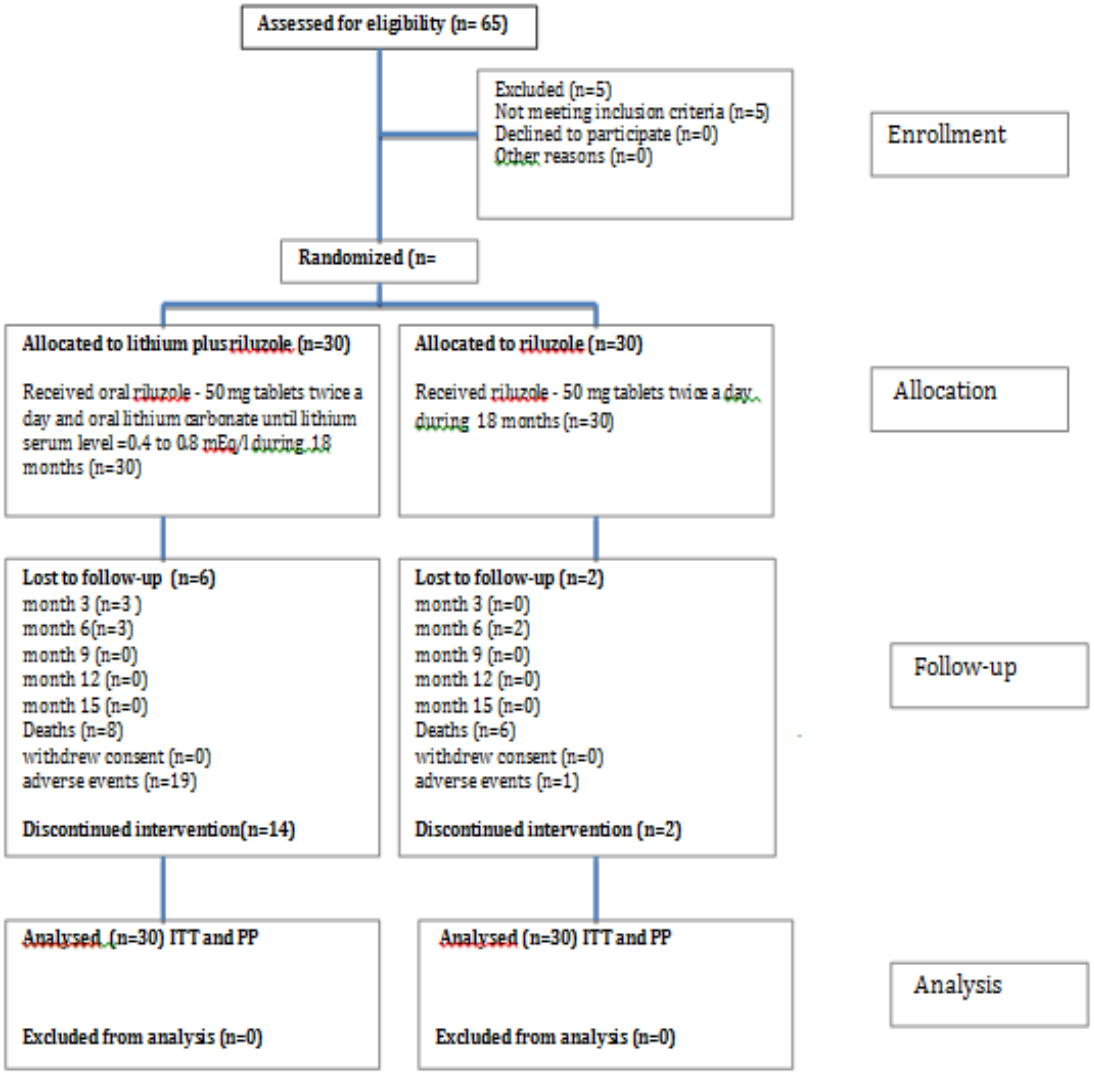

Figure: 1. CONSORT diagram. Allocations, interventions, follow-up and analysis of 60 subjects with amyotrophic lateral sclerosis.

All 30 patients who were randomly assigned to the lithium plus riluzole group had at least one blood lithium concentration measurement between 0.4 to 0.8 mEq/l. The mean blood lithium concentration was 0.7 mEq/l in the experimental group and 0.6 mEq/l in the control group.

According to El Escorial criteria, in experimental group, 23 patients (80%) had definite or probable ALS and in control group, 26 patients (86.7%). The factors that influence survival did not differ between the groups (table 1).

Table: 1. Baseline demographic and clinical characteristics* of subjects. RCT with lithium associated to riluzole versus riluzole in ALS patients. Brasilia, Brazil. 2014-2015.

Characteristics	Lithium+riluzole (n=30)	Riluzole (n=30)	p-value
Mean age±SD (years)	55.1±11.6	57.8±10.3	0.35
Male (%)	18(60)	18(60)	1.0
Caucasian (%)	26(86.7)	25(83.3)	0.08
Bulbar onset (%)	6(20)	9(30)	0.55
Mean symptom duration±SD (months)	26.6±21.3	39.1±46.1	0.18
Mean ALSFRS±SD	27.8±7.1	26.2±7.9	0.39
Mean FVC±SD	73.8±20.8	65.8±17.4	0.11
Mean BMI±SD (Kg/m^2)	25.46±4.8	24.72±3.2	0.49
Mean amp median CMAP±SD	2.71±3.34	2.71±2.8	0.99
Mean amp ulnaris CMAP±SD	3.01±2.46	2.91±2.44	0.88
Mean time to 2_{nd} site ±SD(months)	10±11.5	10.9±21.4	0.83
Mean sat O_2±SD	96.5±1.4	95.2±3.5	0.06
Mean MRC±SD	52.9±5.1	52.5±5.7	0.76
Mean neck strenght (MRC)	4.40	4.47	0.74

*ALSFRS: Amyotrophic Lateral Sclerosis Functional Rating Scale; BMI: Body Mass Index; FVC: Forced Vital Capacity; CMAP: Compound Muscle Action Potential; sat: saturation; MRC: Medical Research Council; SD: Standard Deviation; amp: amplitude

By the end of the study, at 18 months or at endpoint time (death or tracheostomy), 10 (33.3%) patients in the experimental group and five (16.7%) patients in the control group had received a gastrostomy. Nine (30%) patients in the experimental group and 11 (36.7%) patients in the control group had received non-invasive ventilation. Fourteen (46.7%) patients in the experimental group and 19 (63.3%) patients in the control group were alive at 18 months.

Twenty-two patients who underwent use of lithium carbonate and riluzole discontinued the medication, eight (26.7%) of them because they considered the drug ineffective and 14 (46.7%) by the occurrence of adverse events. Table 2 lists the main adverse events observed with lithium carbonate.

Table: 2 Adverse events observed in patients with amyotrophic lateral sclerosis in use of lithium carbonate plus riluzole.

N	ADVERSE EVENT
8	HEADACHE
5	NAUSEA AND VOMITING
3	DIARRHEA
3	POOR APPETITE AND WEIGHT LOSS
2	MUSCLE WEAKNESS
2	TREMOR
1	RESPIRATORY FAILURE*
1	INSOMNIA
25	TOTAL

* Reversible when the drugs were discontinued

Most events were considered minor, but one patient developed a serious event, respiratory distress with O_2 saturation below 90%, that ameliorates after discontinuation of lithium carbonate.

The primary outcome, death or tracheostomy, was analyzed by intention to treat (ITT) and additionally by per protocol (PP) analysis, considering the high rate of non-compliant patients. Table 3 shows that in an ITT analysis (figure 2), the survival functions did not differ significantly between groups (Kaplan-Meier, log-rank 0.38). However, when it is observed in a PP analysis (figure 3), where eight patients used lithium plus riluzole, the survival function was better in the control group, with a mean survival of 14.72 months [95% CI 13.19-16.25]), whereas in the experimental group, the mean survival was 10.59 months [95% CI 7.12-14.07] (log-rank 0.04).

Table: 3. Survival analysis* in patients with amyotrophic lateral sclerosis treated with lithium carbonate plus riluzole versus riluzole. RCT with lithium associated to riluzole versus riluzole in ALS patients. Brasilia, Brazil. 2014-2015.

	Lithium + riluzole			Riluzole			log-rank
	N	endpoint(%)	mean survival - months [CI95%]	N	endpoint(%)	mean survival - months [CI95%]	
ITT	30	16(53.3)	13.12 [10.81-15.43]	30	11(36.7)	15.06 [13.43-16.70]	0.38
PP	8	6(75)	10.59 [7.12-14.07]	50	21(42%)	14.72 [13.19-16.25]	0.04

*ITT: Intention to Treat; PP: Per Protocol. Endpoint: death or tracheostomy

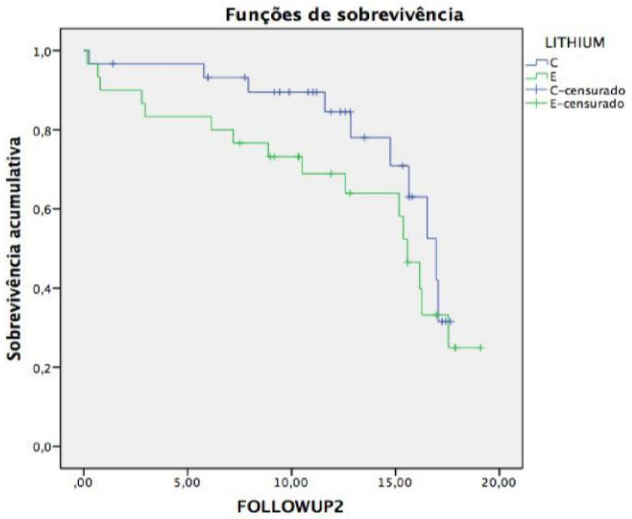

Figure 2. Kaplan-Meier survival analysis in patients with amyotrophic lateral sclerosis treated with lithium carbonate plus riluzole (E) versus riluzole (C). Intention To Treat analysis. Log Rank (Mantel-Cox): 0.38

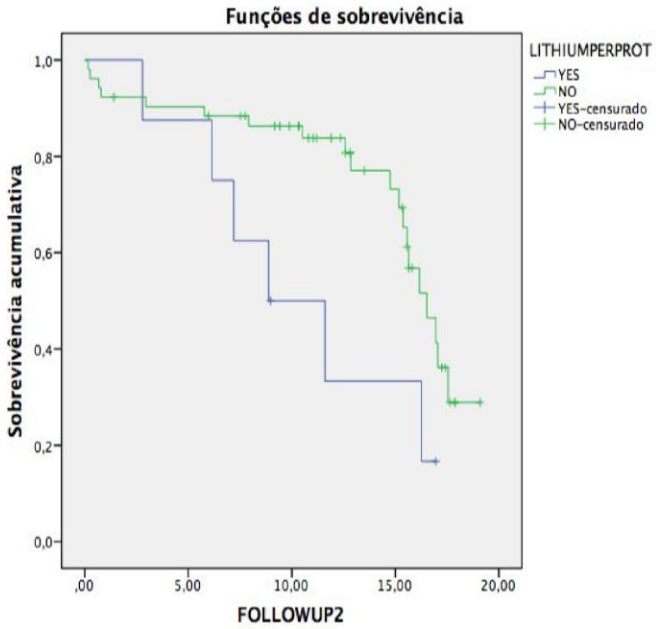

Figure 3. Kaplan-Meier survival analysis in patients with amyotrophic lateral sclerosis treated with lithium carbonate plus riluzole (E) versus riluzole (C). Per Protocol analysis. Log Rank (Mantel-Cox): 0.04

Table 4 shows the mean scores for the secondary outcomes. In an ITT analysis, there were no differences between the groups, related to CFV, MRC scores, ALSFRS and median CMAP area and amplitude. In the unadjusted analysis for the ALSFRS, a functional scale that varies from 0 to 40, the annual rate of change was 8.15±5.24 in the experimental group and 7.36± 4.14 in the control group (p=0.66).

Table: 4. Secondary outcome measures, by time since randomization*. RCT with lithium associated to riluzole versus riluzole in ALS patients. Brasilia, Brazil. 2014-2015.

MEAN VARIATION(SD)	INTENTION TO TREAT			PER PROTOCOL		
	L+R	R	p	L+R	R	p
MRC - 3 MONTHS	1.36(5.54)	2.90(5.76)	0.38	3.14(5.90)	4.31(5.65)	0.62
MRC - 6 MONTHS	3.15(3.68)	3.42(4.85)	0.85	2.80(4.76)	3.35(4.22)	0.79
MRC - 12 MONTHS	4.90(4.39)	4.77(8.10)	0.94	6.50(3.21)	5.88(6.85)	0.87
MRC - 18 MONTHS	10.75(16.76)	10.56(15.66)	0.67	9.56(5.50)	10.55(7.99)	0.88
ALSFRS 3 MONTHS	3.39(2.77)	3.74(3.03)	0.68	2.57(2.15)	3.74(2.98)	0.33
ALSFRS 6 MONTHS	4.84(3.99)	4.35(3.77)	0.71	2.80(3.11)	4.90(3.91)	0.26
ALSFRS 12 MONTHS	8.15(5.24)	7.36(4.14)	0.66	3.50(1.73)	8.48(4.59)	0.04
ALSFRS 18 MONTHS	9.09(4.46)	7.14(3.13)	0.33	6.00(5.66)	8.63(3.91)	0.40
FVC(%) -3 MONTHS	9.00(9.58)	4.42(6.13)	0.81	4.29(4.23)	7.41(8.99)	0.37
FVC (%)- 6 MONTHS	12.09(11.76)	12.73(11.16)	0.83	7.01(8.23)	13.26(11.63)	0.15
FVC(%) - 12 MONTHS	16.63 (16.36))	14.67(18.36)	0.22	23.12(19.90)	20.09(17.54)	0.75
FVC(%) - 18 MONTHS	26.29(19.16)	24.53(14.97)	0.83	14.30(20.22)	26.80(16.45)	0.33
CMAP AREA - 3 MONTHS	2.22(2.15)	4.32(8.44)	0.27	2.26(1.69)	3.41(6.56)	0.63
CMAP AREA - 6 MONTHS	4.22(4.07)	4.23(8.66)	0.16	4.81(2.19)	4.14(6.99)	0.83
CMAP AREA - 12 MONTHS	3.82(4.05)	8.70(13.70)	0.18	6.73(5.13)	6.00(10.56)	0.89
CMAP AREA - 18 MONTHS	5.06(9.64)	6.36(14)	0.86	6.95(13.49)	5.51(11.41)	0.75

L+R: lithium plus riluzole; R: riluzole

*MRC: Medical Research Council; ALSFRS: Amyotrophic Lateral Sclerosis Rating Scale; FVC: Forced Vital Capacity; CMAP: Median nerve Compound Motor Action Potential; SD: Standard Deviation.

However, in a PP analysis, it was observed that the experimental group had a minor slope in ALSFRS at 12 months (3.50±1.73), than in the control group (8.48±4.59) (p=0.04).

Table 5 describes these five patients who had better functional outcome in 12 months by PP analysis. It is observed that lower age at onset, bulbar-onset and a prolonged time from onset of symptoms to diagnosis (above 48 months) were present in three of them.

Table: 5. Patients using lithium and riluzol with better outcome in Per Protocol analysis.

Age (years)	Gender	Time 1	Time 2	Onset	death	tracheostomy	ALSFRS (1)	ALSFRS (12)	BMI
39	M	94	12	spinal (leg)	no	no	33	2	21.5
43	M	48	0	bulbar	no	yes	32	0	24.4
45	M	36	12	spinal (arm)	yes	no	20	1	24.8
56	M	6	5	bulbar	no	yes	32	1	23.0
65	F	72	60	bulbar	yes	no	31	2	22.7

M: male; F: female; ALSFRS: Amyotrophic Lateral Sclerosis Rating Scale (1)= first score; (12)= variation at 12 months; BMI: Body Mass Index; Time 1: onset of symptoms; Time 2: progression to another site.

Analyzing only patients with bulbar-onset, it is observed that those who used lithium carbonate and had higher scores in ALSFRS scale had 46.67±months of disease progression and control patients had 13.56±4.95 months of progression (p=0.031). The variables age, race, FVC, Sat O2, BMI, MRC, neck strenght, CMAP area and amplitude did not showed any significant differences in bulbar-onset patients.

DISCUSSION

In experimental model of ALS, lithium has demonstrated anti-apoptotic and anti-glutamatergic activity[4]; its ability to promote autophagy has been shown in several in-vitro and in-vivo models of neurological diseases.[17] Moreover, lithium used for more than 50 years in the treatment of bipolar disorder and, more recently, cluster headache, is a secure drug because it has well known pharmacokinetics, pharmacodynamics, side-effects, and drug interactions in human beings.[4,8]

However, this study showed that lithium, in combination with riluzole, did not improve survival in patients with ALS and, in addition, showed adverse events so important that reduced compliance and led to interruption of study.

Secondary outcome measures supported the equivalence of lithium and riluzole based upon disease progression, as measured by the ALSFRS, neurophysiological measures, clinical parameters and vital capacity. Additionally, safety analyses revealed a major safety issue and discontinuation of trial medication due to adverse effects that occurred significantly more often in patients taking lithium in comparison with placebo. There is no evidence that treatment with lithium results in an increase in survival at 18 months.

An improvement in functional analysis using ALFRS scale was observed, in some patients with bulbar-onset. However, these patients had important confounding factors, a lower patients age and a significant longer time of disease, which may suggest that they would have a slower progression of the disease.

Lithium has demonstrated neuroprotective effects in cell and ALS animal models. Mice expressing mutant Cu/Zn superoxide dismutase 1 (SOD1) exhibit ALS-like phenotypes, including the formation of intracellular aggregates of SOD1 in the brain and spinal cord, behavioral abnormalities, and premature death.[3,17,18,19] In organotypic slice cultures of spinal cord, chronic treatment with lithium dose-dependently prevents excitotoxic cell death of MNs by inhibiting the GSK-3β signaling pathway.[19]

In 2007, Fornai et al.[3] found that daily doses of lithium that result in plasma levels that range from 0.4 to 0.8 mEq/liter, delay ALS progression in 16 human patients. There were no deaths and few adverse events in the experimental group. In the same study, the authors observed in a genetic ALS animal model, the G93A mouse, a marked neuroprotection following treatment with lithium, which delayed disease onset and duration and augmented the life span.[3] There were no significant differences in baseline variables between Fornai's study and the subsequent trials with lithium.

Recently, a retrospective controlled cohort study with patients with ALS who received autologous bone marrow mononuclear cell transplantation[20] showed neuroprotective effect of lithium with improvement of survival time.

Other larger controlled studies that analyzed the lithium effect in survival and functional progression showed any benefit of lithium[5–7,21], but lower rates of discontinuation by adverse events.

In a large, negative ALS clinical trial (UKMND-LiCALS Study Group et al.[7]) plasma lithium levels were 0.4–0.8 meq/L, barely at the lower therapeutic range for psychiatric disorders and significantly lower than the serum concentration for lithium of its major hypothesized targets (≥1 mM). Indeed, in the antidepressant like rodent model (i.e., the forced-swim test) only plasma lithium levels above 1.3 meq/L significantly reduced immobility-time.[23] Importantly, it has recently been shown[24] that lithium-induced neuroprotection is antagonized by riluzole, thereby suggesting that the drug's neurotoxic effects may mask the potential neuroprotective activity of lithium.[24,18]

In the same study[7], there was no differences in adverse events between experimental and control group (hazard ratio for all serious adverse events 1·14, 95% CI 0·79–1·65) and compliance with treatment was good (65%), but was higher in the placebo group (71%) than in the lithium group (60%).

The important aim of this study was to assess the safety and the adverse events of lithium for the treatment of patients with ALS. The well-known side effects of lithium (e.g., nausea and cephalalgia) occurred more frequently in the lithium treated group. In fact, more patients in the lithium group experienced side effects which led to discontinuation of trial medication, as indicated by another study.[6] Other trials with lithium[5,7,20], except one[22] did not show high rates of discontinuation or adverse events. There was no prior occurrence of respiratory distress with lithium and its mechanism remains unclear.

The narrow therapeutic range of lithium and, therefore, the increased risk of toxicity, it was refrained from including a higher dosage group out of concern for the safety of our patients. The dropout rate for lithium trials tends to be higher compared with trials with other compounds in ALS, which might have obscured the observed effects.

Notably, little is known about potential interactions between lithium and riluzole. However, the limited evidence available suggests a summation effect rather than a detrimental effect of riluzole on the neuroprotective properties of lithium.[24]

There is a large discussion concerning the primary endpoint of the studies and their biomarkers. Early trials focused on mortality, but it is even more difficult to define death and the real time of tracheostomy that is needed, once some patients undergone tracheostomy or gastrostomy and others do not accept the procedures. This study chose, as the primary

endpoint, time to death or tracheostomy because it is a more objective measure and easily comparable to other studies.

As secondary endpoints, the current study focused on clinical markers, such as functional scale ALSFRS, MRC index, respiratory function and neurophysiological biomarkers, such as amplitude and area of CMAP. There was no difference between the two groups.

CONCLUSION

This randomized controlled trial showed low compliance and a high incidence of adverse events of lithium in ALS patients, which resulted in the early termination of the study. We conclude that lithium is ineffective in doses that can be tolerated. The authors discourage future use of the combination therapy riluzole plus lithium.

Author Contribution

MC Moura collected all data. MC Moura, MRCG Novaes and LA Casulari analyzed data, wrote and revised the paper. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

The authors declare no funding. The full trial protocol can be accessed in www.ensaiosclinicos.gov.br

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