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Microdose Lithium Treatment Stabilized Cognitive Impairment in Patients with Alzheimer's Disease

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Abstract: A lower incidence of dementia in bipolar patients treated with lithium has been described. This metal inhibits the phosphorylation of glycogen-synthase-kinase 3-α and β, which are related to amyloid precursor protein processing and tau hyperphosphorylation in pathological conditions, respectively. Following the same rationale, a group just found that lithium has disease-modifying properties in amnestic mild cognitive impairment with potential clinical implications for the prevention of Alzheimer's Disease (AD) when a dose ranging from 150 to 600 mg is used. As lithium is highly toxic in regular doses, our group evaluated the effect of a microdose of 300μg, administered once daily on AD patients for 15 months. In the evaluation phase, the treated group showed no decreased performance in the mini-mental state examination test, in opposition to the lower scores observed for the control group during the treatment, with significant differences starting three months after the beginning of the treatment, and increasing progressively. This data suggests the efficacy of a microdose lithium treatment in preventing cognitive loss, reinforcing its therapeutic potential to treat AD using very low doses.

Keywords: Alzheimer, lithium, GSK-3, Tau, aging, memory.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative illness leading to impairment of memory, language and emotion. The basic ultra-structural lesions that characterize AD and play a critical role in its pathogenesis are the formation of senile plaques, composed of amyloid-β peptide (AB) deposits, and neurofibrillary tangles, formed by hyperphosphorylation of tau-protein filaments. Nowadays, five drugs have been approved for treating AD (galantamine, tacrine, donepezil, rivastigmine and memantine). Unfortunately, these therapies only provide some symptomatic benefit, with variable response among patients, and do not alter the progression of the disease. There is a need to develop new therapies that alter the progression of AD and prevent or slow neurodegeneration. A recent potential strategy to treat AD is the use of lithium, a potent inhibitor of glycogen-synthase-kinase 3-α and β (GSK-3α; GSK-3β), enzymes that may play an important role in the pathogenesis of AD [1-3]. Additionally, a putative neuroprotective effect of lithium [4] plus a lower prevalence of dementia in bipolar humans treated with this metal [5] were observed.

In the elderly, lithium toxicity threshold is lower due to the common increase in fat mass in relation to the lean mass and total water volume, associated to decreased glomerular filtration [6], which makes the usual therapeutic doses of lithium, frequently administered in milligrams, to be toxic. In addition, the increased risk of intoxication due to polypharmacy should be considered [7]. Considering that, this work evaluated the efficacy of a microdose lithium therapy for patients with clinical diagnoses of AD, by the criteria of DSM-IV and NINCDS-ADRDA [8], along 15 months.

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METHODS

Participants

Each participant provided written informed consent. This protocol was approved by our Institutional Human Research Ethics Committee, protocol number 054/07, in accordance with the Brazilian laws, the principles of the Declaration of Helsinki and the Guideline for Good Clinical Practices.

Initially, 113 patients from our Institutional Geriatric Hospital, from both genders, were selected to participate in the study according to the score obtained in the mini-mental state examination (MMSE) [9], a well known test for cognitive level. We selected patients who scored between nine and 21 in the test if they attended school for a maximum of four years and those who scored between 12 and 24 if they attended school for longer than four years [10]. The patients were randomly assigned into two groups, one being "placebo" (n=55), which received the placebo capsule, and the other being "treated", which received the active drug capsule (n=58). Both capsules were indistinguishable. Their mental state was evaluated every three months using MMSE. Along the study, 10 "placebo" and 9 "treated" subjects missed one or more MMSE and were excluded from the statistical analyses of MMSE scores (Table **1**). The MMSE was applied in a double-blinded way by the Physicians of the hospital, i.e.,

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Physicians, patients, family or caregivers were not aware of the patient´s group. According to the Brazilian laws lithium is a controlled drug, therefore the protocol was only open for the principal investigator and to the National Health Surveillance Agency.

Lithium Treatment

The selected lithium dose was 300 µg (40 nmol), administered once daily for 15 months in the form of gluconate or carbonate, and was based on the efficacy of lithium (400µg/day) in improvement of mood [11] and mental state [12], which gives a good drug safety margin to the dose used in this study [6].

Statistical Analysis

As the MMSE score (factors Treatment and Time) does not fill the assumption of the homogeneity of variances according to Levene's test, comparison of means of MMSE scores were done by the Friedman non-parametric test, followed by the Bonferroni test. Gender (Factors Treatment and Gender) were analyzed by two-way ANOVA, followed by Bonferroni test. Prescription drugs were analyzed by chi-square test, a*ge* by Student t- test and time since diagnosis by Fisher's exact test. Data were considered significant when $P<0.05$.

RESULTS

To verify the homogeneity between groups, the last two MMSE applied (once every six months) before the placebo or lithium treatment were retrospectively evaluated, showing no differences between groups, as well as in the Bonferroni test (Fig. **1a**). This data indicates that further differences obtained after starting the treatment should not be due to a bias in MMSE previous performance.

In addition, we found no significant differences related to age, gender (number of males and females), time since the disease clinical diagnosis and other prescribed medication between control and treated groups (Table **2**).

During the study, the treated group showed no decreased performance in the MMSE test, in opposition to the lower scores observed for the control group. The Friedman test showed a significant difference between groups ($P < 0.001$). The Bonferroni test showed that in the MMSE applied just before the beginning of the treatment (trimester 0) no difference was observed between groups, but that was observed in the trimester 1 (control = 17.37 ± 0.86 and treated = 20.60 ± 0.66, $P < 0.01$) and the difference increased along the treatment period (trimester 5: control = 14 ± 1.326 and treated= 19.82 ± 0.9, $P < 0.001$).

In some MMSE tests, some patients were not present, as is shown in Table **1**, and were excluded from the statistical analysis. However it is important to notice that no deaths were registered.

DISCUSSION

Recently, it has been showed that lithium treatment for a year reduced the cognitive decline in amnestic mild cognitive impairment, when compared with placebo, being associated with a significant reduction in CSF concentration of tau protein. These disease-modifying properties were observed

Table 1. Number of Patients Interviewed Before and After the Beginning of the Therapy.

Groups	Before the therapy (Semesters)			After the beginning of therapy (Trimesters)				
	0	1	2	1	2	3	4	5
Control	55	55	55	53	51	47	45	45
Treated	58	58	58	57	57	57	51	49

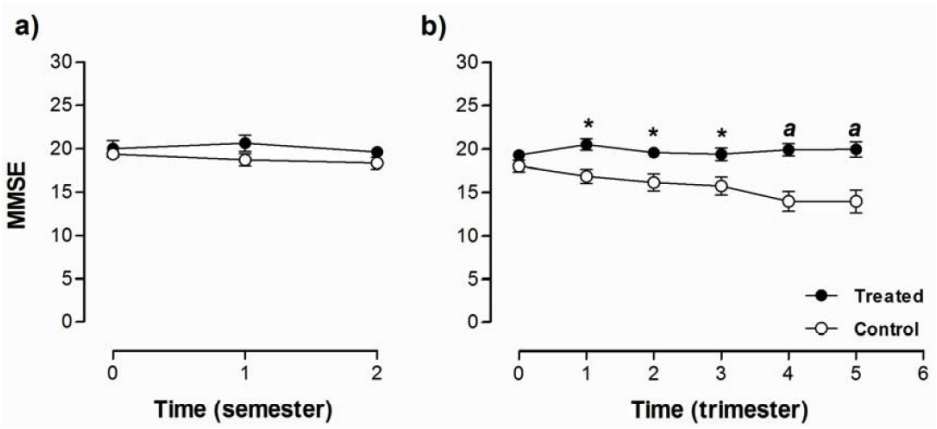

Fig. (1). Scores in the mini-mental state examination (MMSE) of subjects from control or treated groups before placebo or lithium therapy (a) and after the beginning of the therapy (b). The "Semester" 2 in panel "a" corresponds to "Trimester" 0 in panel "b". Data are expressed as means ± S.E.M. Statistical differences are represented by * ($P < 0.01$) and ***a*** ($P<0.001$).

Table 2. Homogeneity Between Number of Subjects Taking Prescription Drugs, Number of Male and Female Subjects (Gender), Age and Time Since Clinical Diagnosis, in Years, Initial MMSE.

	Prescription drugs				Gender		Age	Time since diagnosis		Initial MMSE
	AChE-I	NMDA-I	ECA-I	HypGly	Male	Female		≤6	≥6	
Control	22	46	15	7	14	41	78.00±0.76	47	9	17.95±0.73
Treated	26	45	19	8	24	34	77.00±0.97	48	10	19.48±0.67

Abbreviations: AChE-I, Acetylcholinesterase inhibitors; NMDA-I, N-Methyl-D-aspartic *acid inhibitors; ECA-I,* Angiotensin converter *enzyme* inhibitor, HypGly, Hypoglycemic agents; MMSE-Mini Mental State Exame. Data were considered significant when $P<0.05$.

when a dose ranging from 150 to 600 mg was used, being safe and well-tolerated [13]. In the present work we used a dose about 1000 times lower than the dose described above, which promoted stabilization of cognitive impairment in patients diagnosed with Alzheimer's disease. It is important to state that although we do not have lithium serum levels registration, after 15 months of treatment patients did not complain or show any kidney or thyroid dysfunction or any other organic disturbance that could be caused due to toxic events of a lithium microdose treatment.

The observed effects can be related to cell survival leading to modulation of long-term potentiation (LTP), which is a wholly accepted model for the long-term memory keeping [14, 15]. It has already been shown that treatment of rats or humans with therapeutic doses of lithium induced neuronal plasticity related to LTP [16, 17]. Although these doses were higher than the one used in this study, the effects were related to the inhibition of glycogen synthase kinase 3 (GSK-3) activity, which is a postulated molecular action mechanism for lithium salts [18-20].

The enzyme GSK3 has two isoforms, namely GSK-3α and GSK-3β. GSK-3α can increase the production of amyloid-β peptides, through the cleavage of amyloid precursor protein (APP). On the other hand, the GSK-3β has a small participation in this process [3]. Also, the increase in amyloidal deposition promotes Tau protein phosphorylation by GSK-3α and β, through protein kinase C inactivation, leading to the formation of paired helicoidal filaments, another important marker of AD [21]. The enzymatic activity of GSK-3 can be inhibited by protein kinase B and other kinases which can phosphorylate inhibitory sites located in serines 21 (GSK-3α) and 9 (GSK-3β) [1, 22].

The main mechanism leading to the neuroprotective effects of lithium involves the inhibitory phosphorylation of these serines (21 and 9) leading to the inhibition of GSK-3α and β (18) and by competing with magnesium, which is important for transferring the phosphoryl to the substrate (19), changing the GSK-3 conformation and blocking their link to the substrate (20). GSK-3 is also involved in the neuroinflammation associated with AD. In this way, it was shown that GSK-3β increases tumor necrosis factor-α production and its inhibition could be a potential target for anti-inflammatory intervention [23].

CONCLUSION

Although this is a small study the current data suggests, for the first time, the effectiveness of lithium microdoses in diminishing the cognitive decline observed in AD patients and can be a promising formulation for the treatment of this disease. Using only MMSE as an outcome variable was a limitation of the study and the use of other clinical trials will be addressed in the future. Nowadays, protocols using APP transgenic mice are in progress in our laboratory to test the efficacy and safety of lithium at this dose, starting in young mice and following them until they become old.

AUTHOR CONTRIBUTIONS

MAN and HSB designed the experiments. MAN generated data. All authors analyzed data, discussed the results, wrote and commented on the manuscript.

COMPETING FINANCIAL INTERESTS

The data obtained in this study is the subject of a patent application concerning a possible treatment for Alzheimer's disease (Pub. No.: WO/2011/123916).

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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