Journal of Alzheimer's Disease xx (2021) x–xx
DOI 10.3233/JAD-201501
IOS Press

Interventional Study to Evaluate the Clinical Effects and Safety of the Nutraceutical Compound BrainUp-10® in a Cohort of Patients with Alzheimer's Disease: A Multicenter, Randomized, Double-Blind, and Placebo-Controlled Trial

Leonardo Guzman-Martinez[a], Gonzalo A. Farías[b,c], José P. Tapia[a], María P. Sánchez[d], Patricio Fuentes[b], Sergio Gloger[e] and Ricardo B. Maccioni[a,b,*]

[a]*Laboratory of Neuroscience and Functional Medicine, International Center for Biomedicine (ICC) and Faculty of Sciences University of Chile, Santiago, Chile*
[b]*Department of Neurology and Center for Advanced Clinical Investigation (CICA), Faculty of Medicine University of Chile, Santiago, Chile*
[c]*Center for Advanced Clinical Investigation (CICA), Faculty of Medicine University of Chile, Santiago, Chile*
[d]*Faculty of Chemistry and Biology University of Santiago of Chile, Santiago, Chile*
[e]*Biomedica Research Group, Santiago, Chile*

Accepted 18 March 2021

Abstract.

Background: Clinically-evaluated nutraceuticals are candidates for Alzheimer's disease (AD) prevention and treatment. Phase I studies showed biological safety of the nutraceutical BrainUp-10®, while a pilot trial demonstrated efficacy for treatment. Cell studies demonstrated neuroprotection. BrainUp-10® blocks tau self-assembly. Apathy is the most common of behavioral alterations.

Objective: The aim was to explore efficacy of BrainUp-10® in mitigating cognitive and behavioral symptoms and in providing life quality, in a cohort of Chilean patients with mild to moderate AD.

Methods: The was a multicenter, randomized, double blind, placebo-controlled phase II clinical study in mild to moderate AD patients treated with BrainUp-10® daily, while controls received a placebo. Primary endpoint was Apathy (AES scale), while secondary endpoints included Mini-Mental State Examination (MMSE), Trail Making Test (TMT A and TMT B), and Neuropsychiatry Index (NPI). AD blood biomarkers were analyzed. Laboratory tests were applied to all subjects.

Results: 82 patients were enrolled. The MMSE score improved significantly at week 24 compared to baseline with tendency to increase, which met the pre-defined superiority criteria. NPI scores improved, the same for caregiver distress at 12th week ($p = 0.0557$), and the alimentary response ($p = 0.0333$). Apathy tests showed a statistically significant decrease in group treated with BrainUp-10®, with $p = 0.0321$ at week 4 and $p = 0.0480$ at week 12 treatment. A marked decrease in homocysteine was shown with BrainUp-10® ($p = 0.0222$).

*Correspondence to: Ricardo B. Maccioni, MD, PhD, International Center for Biomedicine, Avda. Vitacura 3568, D511-512, Vitacura, Santiago, Chile. E-mail: rmaccioni45@gmail.com.

ISSN 1387-2877/$35.00

Conclusion: Data show that BrainUp-10® produces a statistically significant improvement in apathy, ameliorating neuropsychiatric distress of patients. There were no compound-related adverse events. BrainUp-10® technology may enable patients to receive the benefits for their cognitive and behavioral problems.

Keywords: Alzheimer's disease, blood biomarkers, BrainUp10®, clinical trial, nutraceutical compound

INTRODUCTION

Alzheimer's disease (AD) and related dementias are increasing their prevalence as world population is increasing their life expectancy. These diseases are complex and related to multiple risk factors, including genetic load, sex, and age, but also to many modifiable risk factors including low education, hypoacusis, social isolation, and cardiovascular risk factors [1]. Across several years, the pharmaceutical industry has been struggling to deliver new, innovative treatments pointing to specific molecular targets, including immunotherapies against misfolded, aggregated proteins like amyloid-β and tau, but in spite of multiple rationally directed efforts, no real disease-modifying drugs have proven efficiency [2]

Many researchers have proposed that it may not be possible to develop an effective drug against AD and other forms of dementia, based on a single drug-single target approach since there are so many multiple factors triggering neurodegeneration at the same time, and most importantly, pure diseases appear to be a minority since mixed pathology can be found in most cases in anatomopathological series. As a response, multitarget directed nutraceutical compounds appear as an appropriate response to supplement vitamins and necessary dietary compounds, improve redox balance, diminish neuroinflammatory responses, and control protein aggregations, in order to stop neurodegenerative processes and favor a neuroprotective ambient in the brain [3]. Besides, behavioral alterations reach 80–90% of prevalence in different stages of AD [4, 5], with the apathy the most common of these symptoms [6]. Thus, it has been documented that apathy, defined as the loss of interest or motivation, increase it frequency through the progress of disease, from 42% in early stages to 90% in severe AD [6]. Apathy has been associated with great functional and cognitive impairment in patients with AD [7], representing an important distress factor in caregivers. This directly impacts the quality of life of both the patient and the caregiver, which implies a greater emotional burden, increases the number of hospitalizations, and adds a profound economic impact on society. Furthermore, apathy has been highly associated to mortality in patients with AD [8]. Methylphenidate has shown slight improvement in apathy [9]; however, there is no approval pharmacologic treatment for apathy in AD and other dementias.

Brain Up-10® (BU-10) is an innovative nutraceutical formula of selected concentrations of B complex vitamins, i.e., pyridoxal phosphate, folate, and cyanocobalamin, with the impressive antioxidant properties of fulvic acid and other components of the natural product *Andean shilajit,* an endemic fossilized natural product found in the Andes mountains in the north of Chile [2, 6]. BU-10, which show beneficial neuroprotective effects, was developed by an experienced research group headed by Prof. Dr. Ricardo Maccioni and was tested *in vitro*, in animal models, and in humans in a phase I study, demonstrating to be clinically safe and effective in reducing key pathogenic processes like tau aggregation [3, 10, 11]. In a pilot phase II trial, the formula has also shown a clinical improvement in behavior, apathy, and levels of aggregated tau in peripheral blood platelets biomarker [11]. These promising results together with the relevance of mitigating behavioral symptoms for AD patients and caregivers led us to perform a double blind, randomized, placebo-controlled phase II trial—the BU10/II clinical trial—to determine the clinical efficacy of BU-10 to improve behavior and cognition in subjects with clinical diagnosis of mild to moderate AD. Our hypothesis is that the BU-10 nutraceutical is safe and effective in mitigating some cognitive and behavioral symptoms of AD in mild to moderate stage patients compared to placebo intervened group. The objective of this protocol was to evaluate the efficacy of BU-10, compared to placebo, using neuropsychological instruments for the general cognitive performance and with special emphasis in motivation, attention, and memory, in subjects with mild to moderate AD. Apathy was selected as our primary endpoint since the most notorious effect described by patients and caregivers, in previous research, was an increased energy and motivation for daily tasks. The secondary objectives of this study were the evaluation of the effects of BU-10 in other clinically significant domains, including the behavior and quality of life

and to assay the safety and tolerability of BU-10, in relation to placebo, in subjects with mild to moderate AD.

METHODS

Study design

The BU-10/II clinical trial was a multicenter, randomized, double-blind, placebo-controlled parallel group study to evaluate the efficacy and safety of 24 weeks of treatment with BU-10 in comparison to a placebo control group. We established a selection period of up to 28 days followed by 24 weeks of treatment with BU-10 or placebo randomized 1:1. Efficacy and safety evaluations were conducted at baseline (day 1), week 4, week 12, and week 24 or early termination, in each of the two clinical centers of the multicentric study.

Participants

A total of 82 patients were selected according to inclusion/exclusion criteria. Subjects enrolled for the study were males and females between the ages of 55 and 85 years, diagnosed with AD based on the DSM.IV-TR and the NINCDS-ADRDA criteria. 74 subjects competed the whole study as indicated in Fig. 1 (consort flow diagram). Brain imaging consistent with a diagnosis of probable AD (magnetic resonance imaging (MRI) or computed tomography (CT), if the MRI was not feasible in the opinion of the

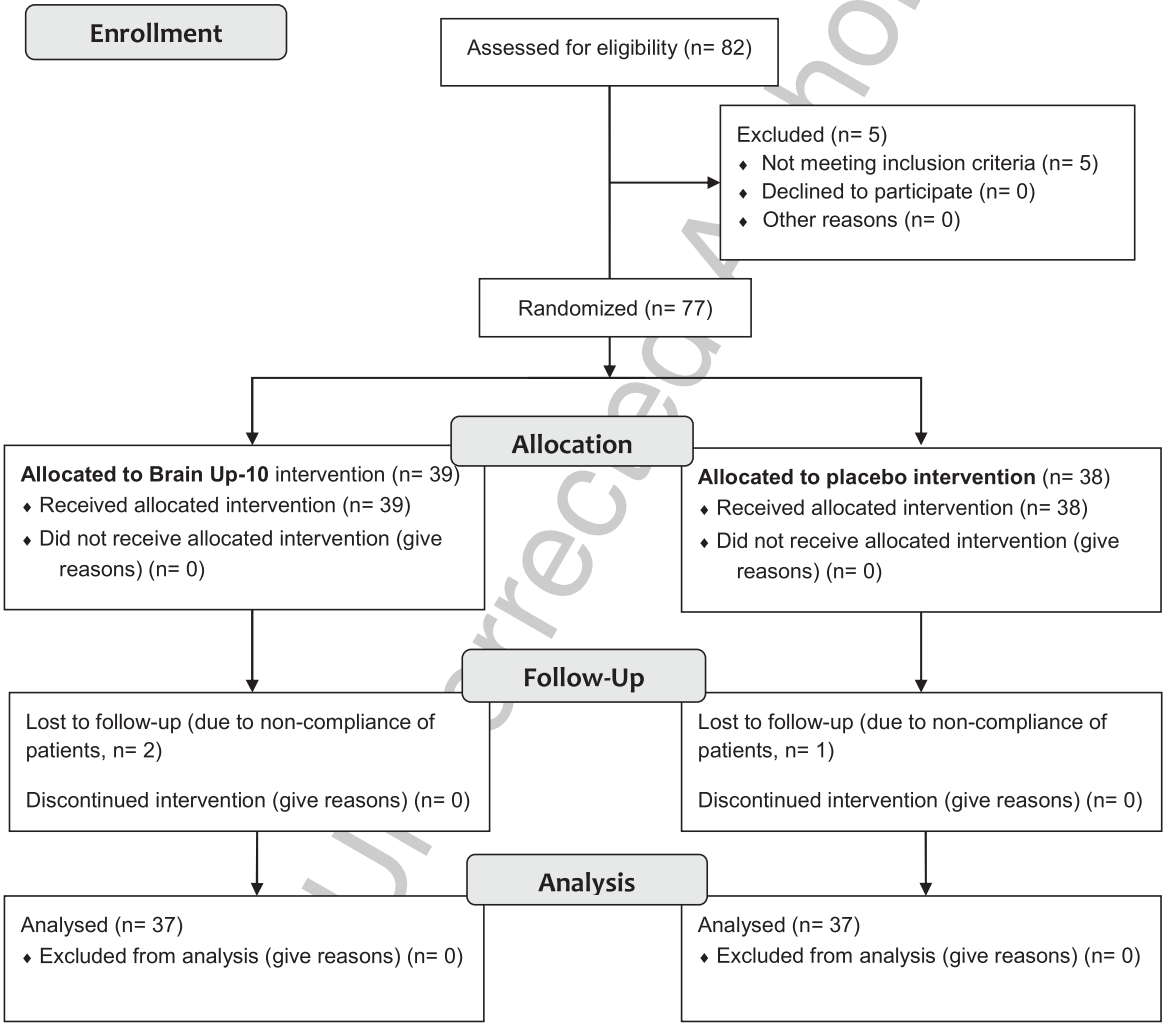

Fig. 1. CONSORT flow diagram showing participant enrollment, allocation, follow up, and status in trial and analysis.

investigator) was required to be performed in the last 12 months. Patients with MRI or CT that indicate cortical infarction, strategically located subcortical gray matter infarction (i.e., hippocampus, thalamus), multiple lacunae or extensive abnormalities in the white matter were excluded. Refer to the Supplementary Material to review the complete inclusion/exclusion criteria.

Sequence and allocation

Subjects were recruited at Biomedica Research Group in Santiago, Chile and Psicomed in Antofagasta, Chile, and were randomized to treatment within each site, assigning an identification number for each subject in the screening visit. The Investigators obtained subject identification numbers and the allocation of the study drug using an administration drug system based on the Interactive Web Response System (IWRS).

Randomization

Subjects were randomized in a 1:1 ratio to BU-10 or placebo through an IWRS that assigned an identification number to each individual, maintaining the blind with the use of codes. Only after the end of the study was the randomization list disclosed for the analysis of results.

Study intervention

This study was designed as a double-blind, parallel-group trial in which subjects received a 24-week intervention with BU-10 or placebo. The protocol and all documentation of the study were approval by the corresponding Institutional Review Board, the Servicio de Salud Metropolitano Oriente (SSMO), and the Metropolitan Health Service SEREMI of Santiago, as well as by the Medical Ethics Committee of the University Hospital. After signing the informed consent form previously approved by SSMO, subjects in the BU-10 treatment were given bottles containing 300 mg capsules according to the allocation by the randomization system. Subjects in the placebo intervention group were given similar-looking bottles with 300 mg capsules containing the placebo treatment. At the baseline, week 4, and week 12 visits of the study, enough trial medication was provided to complete the dosage until the next scheduled visit. The subjects took the study medication under the supervision of the caregiver.

The study medication was ingested orally once a day, in the morning, in a dose of two 300 mg capsules (daily dose of 600 mg), starting the day after the baseline visit. The study medication was taken with water and ingested with or without food.

Compliance

A data booklet was given to each subject to record each dose. This booklet was provided to the research center for assessment at each visit. At each clinic visit, the subject returned the medication from prior treatment periods to the investigator. Compliance with medication was calculated as follows: divide the total number of tablets taken by the number of prescribed tablets. This number was then multiplied by 100 to get the percentage of compliance. Center staff checked the booklet to assess whether there was a match between the daily booklet information and the returned tablets. Dosage records contained details of tablets taken, forgotten, or lost for an accurate measure of compliance. If compliance was less than 80% or greater than 120% in any visit, reasons were immediately registered. Care was taken that subjects must not make up for forgotten doses. If there are two consecutive visits in which compliance is less than 80% or greater than 120% for the study drug, the subject was discontinued from the study for noncompliance after discussing it with the medical monitor.

Concomitant medication(s)

A list of prohibited and permitted concomitant medications were defined. These lists were provided as guidelines of the medications likely to affect cognition or behavior. Prohibited drugs, prescribed or not, cannot be taken within 28 days or 5 half-lives (whichever is longer) prior to the screening visit and throughout the trial (except as agreed by the sponsor). The use of donepezil, galantamine, rivastigmine, and related cholinesterase inhibitors, and memantine were permitted as long as the dose is stable for at least 90 days prior to baseline visit and during the 24-week study. None of subjects modified their previous baseline treatment to avoid bias of possible interference effects by other active substances. Medications taken after the first dose of trial medication were recorded as concomitant medication. All concomitant medications taken during the trial must be recorded. All subjects were asked about the concomitant medication in each clinical visit.

Biochemical markers supplement evaluation

This study included an investigative additional component, which involves the collection of biological samples for blood biomarker analysis involving the measurement of tau protein and amyloid-β protein precursor (AβPP) in platelets, according to Neumann et al. (2011) [12] and Borroni et al. (2001) [13]. They were analyzed at the initial visit and in the last visit (week 24).

Clinical examination and laboratory tests

Patients (BU-10 treated and placebo groups) were subjected to a battery of laboratory tests in addition to the full physical and neurological examination, in order to assess the complete clinical conditions of every patients and control subjects. Laboratory exams included complete urine tests, biochemical profiling from blood samples, blood count, determinations of vitamins B12 and folate in blood, and homocysteine blood levels. These were measured at the initial visit and in the last visit in week 24.

Outcome measures

The main statistical outcome was to estimate the effect of BU-10 through the 24-week treatment period using the Apathy Evaluation Scale (AES) [14, 15], clinician and informant versions, and for the co-primary ADAS-cog [16], Mini-Mental State Examination (MMSE), quality of life five dimensions, three levels (EQ-5D-3L) test, and Trail Making Test (TMT A and TMT B) [17, 18]. The primary significance test evaluated the null hypothesis, that the mean difference in the apathy tests scores between active and control groups at 24 weeks, was less than or equal to zero. The primary significance test was $\alpha = 0.05$ (one tail type 1 error). The main reason to define apathy as the primary outcome derived from preliminary observation in a "survey" study that suggested that apathy was a primary symptom modulated by BrainUp-10®. The present clinical study has been important to quantitatively assess BrainUp-10® efficacy in mitigating apathy.

The secondary outcomes assessed were the mean difference between the MMSE [19], the Neuropsychiatric Inventory (NPI) [20] questionnaire, direct and indirect digit test, and the serial sevens subtraction test. The level of significance considered was $\alpha = 0.05$. In addition, the Scale EuroQol-5d that evaluate five aspects of the quality of life was also assessed in all patients [21].

Sample size determination

Regarding the co-primary assessment criteria, it has been considered that this sample size will allow detecting differences of up to 2.0 points on the AES [14, 15] with a level of significance of $\alpha = 0.05$ (one sided) and a power of 80%. The standard deviation used in the calculation was 3.4, a value consistent with the results of a pilot study carried out by our research team that showed a clinical effect in 93% of the subjects who received the experimental treatment.

Each group should have 36 patients; therefore, the total sample should have a total size of 72 patients. A greater percentage of the sample was considered due to the possible losses.

Statistical methods

Data were reported as mean ± SD or n (%). The Fisher's Exact Test or Pearson's chi-squared test was used for testing statistical differences between categorical data (co-variables). To test differences between two groups, the unpaired Student's *t*-test was used for data with normal distributions and the non-parametric Wilcoxon rank-sum or Mann-Whitney U Test was used for data with a non-normal distribution. Statistical analyses were performed with Statistical Software STATA version 13.1 (StataCorp, College Station, TX, USA). The significant level considered in this study was $\alpha = 0.05$, (p-values <0.05 were considered statistically significant).

RESULTS

A total of 82 patients were screened for eligibility, of which 77 met the criteria, and 3 out of 77 were lost during the study due to non-compliance. Thus, complete information of 74 subjects was available for analysis as described in Consort flow diagram (see Fig. 1). Demographics and relevant clinical information are displayed in Table 1. No significant demographics nor randomization differences were found between the two screening sites, both in Santiago (Biomedica) and Antofagasta (Psicomedica), Chile. Placebo and BU-10 groups were similar in most demographical characteristics. Only a slight difference was found in the frequency of diabetes (Table 1). Laboratory values were also very similar at time 0 between both groups, but there was a significant difference in plasma homocysteine between BU-10 and placebo ($p = 0.022$) with a reduced homocysteine in BU-10 group with value of 15.98 ± 4.04

Table 1
Demographic features and relevant clinical data from the different intervened groups

	BU-10 intervened group	Placebo intervened group	*p*
Screening Site 1 – n (%)	21 (56.8)	22 (59.5)	
Screening Site 2 – n (%)	16 (43.2)	15 (40.5)	
Mean Age – (SD)	73.0 (7.1)	73.9 (5.9)	0.5550*
Gender – n (%)			
Feminine	26 (70.27)	27 (72.97)	0.7970#
Masculine	11 (29.73)	10 (27.03)	
Education – n (%)			
Incomplete secondary education	6 (16.2)	4 (10.8)	–
Complete secondary education	12 (32.4)	9 (24,3)	
Incomplete High school	7 (18.9)	9 (24.3)	
Complete High school	7 (18.9)	5 (13.5)	
Technical education incomplete	0 (0)	2 (5.4)	
Technical education complete	3 (8.1)	7 (18.9)	
Incomplete University education	1 (2.7)	0 (0)	
Complete University education	1 (2.7)	1 (2.7)	
High blood pressure			
Yes – *n* (%)	10 (27.3)	22 (59.5)	0.4690#
No – *n* (%)	27 (72.9)	15 (40.5)	
Diabetes mellitus			
Yes – *n* (%)	5 (13,5)	11 (29.7)	0.1570§
No – *n* (%)	32 (86,5)	26 (70.3)	
Peptic ulcer			
Yes – *n* (%)	0 (0)	0 (0)	–
No – *n* (%)	37 (100)	37 (100)	
Hypothyroidism			
Yes – *n* (%)	10 (27.0)	7 (18.9)	0.5810§
No – *n* (%)	27 (73.0)	30 (81.1)	
Hyperlipidemia			
Yes – *n* (%)	16 (43.2)	15 (40.5)	0.8140#
No – *n* (%)	21 (56.8)	22 (59.5)	
Chronic obstructive pulmonary disease			
Yes – *n* (%)	0 (0)	2 (5.4)	–
No – *n* (%)	37 (100)	35 (94.6)	
Coronary heart disease			
Yes – *n* (%)	2 (5.4)	0 (0)	–
No – *n* (%)	35 (94.6)	37 (100)	
Rhinitis			
Yes – *n* (%)	0 (0)	0 (0)	–
No – *n* (%)	37 (100)	37 (100)	
Stroke History			
Yes – *n* (%)	0 (0)	0 (0)	–
No – *n* (%)	37 (100)	37 (0)	
Asthma			
Yes – *n* (%)	0 (0)	2 (5.4)	–
No – *n* (%)	37 (100)	35 (94.6)	

*Mann–Whitney U test; #Pearson's chi-squared test; §Fisher's exact test.

mM as compared with placebo with a value of 18.17 ± 4.89 mM at week 24, while homocysteine levels were stable in the BU-10 group between time 0 (15.95 ± 4.90 mM) and week 24 (15.98 ± 4.04 mM). No differences in plasma B12 vitamin or folate were found between groups in the 24 weeks period (Table 2).

When we evaluated the primary end point and made a sub-analysis of responses to the questionnaire we found in the BU-10 group a clear tendency to "start things on their own" more frequently and with higher autonomy as compared with the placebo controls. However, we found no significative differences in the clinician version of the AES, i.e., the clinician impression of apathy symptoms during clinical interview (Fig. 2A, Table 3).

In the informant-reported version of AES, we found statistically significantly lower apathy scores of the BU-10-treated group at week 4 ($p = 0.0275$;

Table 2
Biochemical data, participant's clinicals exams summary

Clinical exam	BU-10 intervened group $n=37$				Placebo intervened group $n=37$				
N = 74	Mean	Std. Dev.	Min	Max	Mean	Std. Dev.	Min	Max	*p*
Initial Blood Glucose	98.38	15.01	84.00	169.00	105.24	25.42	79.00	185.00	0.3301
Blood Glucose, 24 weeks	102.19	18.50	85.00	169.00	106.68	38.36	67.00	272.00	0.4587
Initial Glycemia urine	0.00	0.00	0.00	0.00	0.25	1,51	0.00	9.20	-
Glycemia urine, 24 weeks	0.00	0.00	0.00	0.00	0,06	0.35	0.00	2.15	-
Initial Homocysteine	15.95	4.90	9.80	33.20	15.58	5.23	7.90	32.80	0.5776
Homocysteine, 24 weeks	15.98	4.04	10.5	27.30	18.17	4.89	6.70	30.10	**0.0222***
Initial B12 Vitamin	460.54	356.26	198	2.000	519.14	371.02	215	1,875	0.1599
B12 Vitamin 24 weeks	515.14	270.82	199	1.395	500.86	384.43	190	2000	0.2118
Initial Folic Acid	12.98	6.04	3.50	37.10	12.42	5.54	3.6	33	0.8544
Folic Acid, 24 weeks	18.85	11.39	2.50	7.50	29.62	113.85	0.00	703	0.8544

*Wilcoxon rank-sum test/Mann-Whitney U Test.

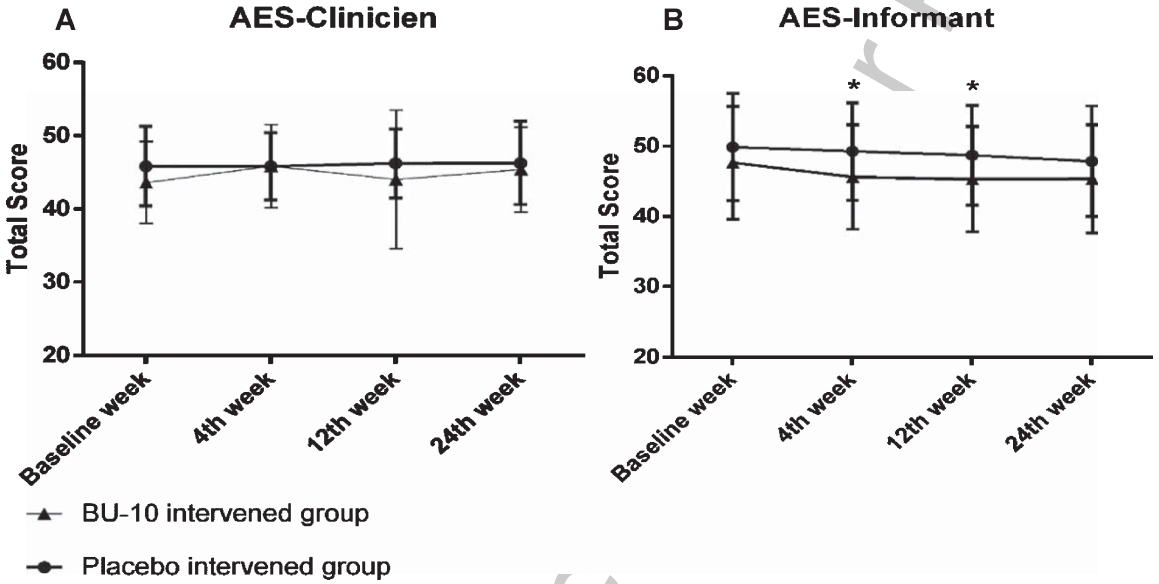

Fig. 2. The Apathy Evaluation Scale (AES) was used as an objective and reliable measure of decreased motivation in patients with dementia. It is an 18-item scale, which allows the evaluation of the behavioral, cognitive, and emotional aspects of apathy and takes 10 to 20 minutes to administer. In (A), the results obtained by the clinical professional are observed, in which there is no difference in each study group. However, when analyzing the reported version (B) by the patient's caregiver, we found significantly lower apathy scores in the BU-10 treated group at week 4 ($p=0.0321$; Student's *t*-test) and week 12 ($p=0.0480$; Student's *t*-test) compared to the placebo intervened group.

Student *t*-test) and week 12 as ($p=0.0378$; Student *t*-test) compared with placebo counterpart (Fig. 2B, Table 3). Besides, in the analyses of specific responses we found a significant increase in the frequency of "interest in things" and "get things done during the day" for the BU-10 group as compared with placebo.

We further evaluated neuropsychiatric symptoms with NPI. Two symptoms are involved in NPI evaluation: distress and delusion. We did not find statistically significant differences between groups in total NPI scores. When we evaluated data from each domain of NPI, we found significant differences with lower scores in symptoms of delusions (Fig. 3A) and caregiver distress due to this symptom (Fig. 3B) as compared with the placebo data (Table 3). We have to point out that the distress analysis of NPI is based the caregiver's interviews. This may explain why at 12 weeks we found a highly significant effect of BrianUp-10®, and that these differences decrease at 24 hours. Caregivers noticed improvement in their patient's behavior at 12 weeks; however, at 24 weeks, their perception may change since the results do not satisfy their expectations. In the caregiver interviews, evidently emotional aspects play a role.

table 3
Summary table of the results obtained with the neuropsychological studies

Scale ($N = 74$)	BU-10 intervened group Mean Score (SD)	Placebo intervened group Mean Score (SD)	p
AES-Clinician			
Baseline week	43.59 (5.4)	45.87 (5.4)	0.0718#
4th week	45.86 (5.7)	45.84 (4.6)	0.9137#
12th week	44.03 (9.4)	46.24 (4.7)	0.2051*
24th week	45.38 (5.8)	46.29 (5.7)	0.321#
AES-Informant	*Mean Score (SD)*	*Mean Score (SD)*	
Baseline week	47.65 (8.0)	49.89 (7.6)	0.2209*
4th week	45.62 (7.4)	49.27 (6.9)	**0.0321***
12th week	45.29 (7.5)	48.70 (7.1)	**0.0480***
24th week	45.35 (7.7)	47.87 (7.8)	0.3062#
Mini-Mental State Evaluation	*Mean Score (SD)*	*Mean Score (SD)*	
Screening	20.76 (3.3)	20.73 (3.4)	0.911#
Baseline week	21.2 (3.3)	20.41 (4.0)	0.338#
12th week	21 (3.8)	20.14 (4.2)	0.437#
Hachinski Ischemia Score	*Mean Score (SD)*	*Mean Score (SD)*	
Baseline week	1.89 (1.08)	2.05 (1.18)	0.538*
Trail Making Test A	*Mean Score (SD)*	*Mean Score (SD)*	
Baseline week	126.10 (78.1)	150.60 (87.1)	0.2056*
12th week	126.20 (67.3)	137.20 (74.4)	0.5028*
24th week	127.90 (96.2)	132.50 (71.1)	0.8159*
ADAS-COG	*Mean Score (SD)*	*Mean Score (SD)*	
Baseline week	19.76 (6.7)	21.28 (6.9)	0.3278#
12th week	20.09 (7.1)	20.66 (6.6)	0.7416#
24th week	19.95 (7.8)	20.84 (7.8)	0.7553#
Neuropsychiatric Inventory			
Total Score	*Mean Score (SD)*	*Mean Score (SD)*	
Baseline week	12.86 (9.0)	16.70 (11.4)	0.1378*
12th week	10.16 (7.1)	14.50 (14.0)	0.2696*
24th week	11.05 (9.6)	12.70 (11.2)	0.5735*
Neuropsychiatric Inventory			
Caregiver Distress Score	*Mean Score (SD)*	*Mean Score (SD)*	
Baseline week	11.92 (8.7)	13.40 (8.3)	0.4354*
12th week	9.51 (8.3)	12.50 (8.3)	0.0557*
24th week	10.51 (9.2)	9.60 (7.8)	0.8241*
EQ-5D-3L	*Mean Score (SD)*	*Mean Score (SD)*	
Baseline week	70.51 (15.16)	65.27 (21.5)	0.3796#
4th week	74.46 (14.06)	67.68 (15.06)	0.0695#
12th week	73. 81 (14.44)	72.11 (19.92)	0.9548#
24th week	75.59 (15.16)	75.03 (17.78)	0.9634#
Tau Biomarker	*Mean Score (SD)*	*Mean Score (SD)*	
Baseline week	1,60 (0.67)	1.59 (0.60)	0.6637*
24th week	1.44 (0.47)	1.39 (0.45)	0.6401*
APP Biomarker	*Mean Score (SD)*	*Mean Score (SD)*	
Baseline week	3,82 (3.72)	3.40 (3.56)	0.7126#
24th week	3.43 (2.77)	3.69 (2.76)	0.8811#

#Wilcoxon rank-sum test/Mann–Whitney U test; *Student's t-test.

Regarding cognitive evaluations, we found no differences between groups in ADAS-Cog during the 24-week period. We also did not find significant differences in MMSE scores, but a tendency for increased stability was appreciated during the 24-week period, between placebo and BU-10 group (Fig. 4 and Table 3).

Quality of life was evaluated with five dimensions, three levels (EQ-5D-3L) test, using the EuroQOL-5d questionnaire. Although no significant differences between groups were found in total scores (Table 3), differences between the BU-10 treated group with respect to placebo were found at the week 4 (Table 3). Besides, a significant improvement in alleviating mobility problems (stay in bed) were found at week

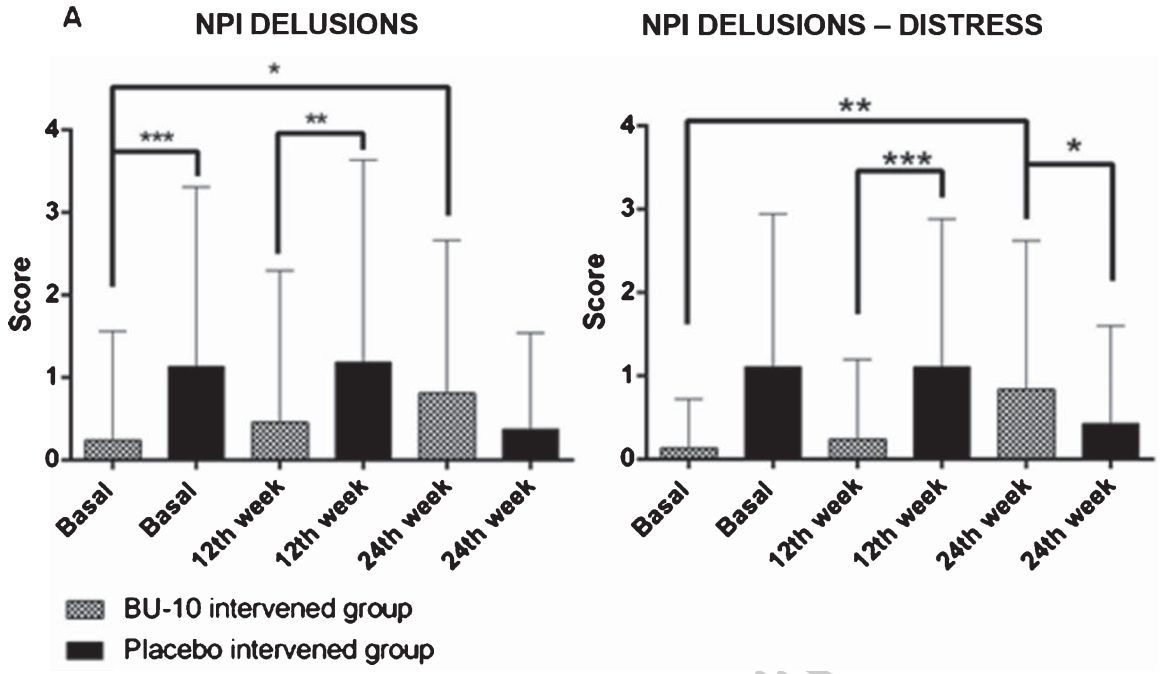

Fig. 3. The Neuropsychiatric Inventory (NPI) focuses on the evaluation of non-cognitive symptoms in patients with dementia. It allows the evaluation and follow-up of the neuropsychiatric disorders of these patients by rating 12 items that must be evaluated through a semi-structured interview. The frequency and intensity of each symptom explored is considered to establish the final score. A) shows the NPI scale related to delusions which presents a significant difference between the baselines of both study groups with a $p = 0.0035$ (Wilcoxon rank-sum test/Mann-Whitney U Test), between the results obtained at the beginning of the study and 24 weeks for the BU-10 intervened group ($p = 0.0082$, Wilcoxon matched-pairs signed-rank test) and when comparing both study groups at week 12 ($p = 0.0069$; Wilcoxon rank-sum test/Mann-Whitney U Test). On the other hand, in (B), the BU-10 intervened study group presented a significant difference between the NPI Delusions -distress values at the start of the study versus at 24 weeks, and this in turn, also presented a statistical significant difference when comparing the BU-10 intervened group at 24 weeks after the start of the study ($p = 0.0144$; Wilcoxon matched-pairs signed-rank test) and also at week 12 ($p = 0.0093$; Wilcoxon rank-sum test/Mann-Whitney U Test) when comparing both groups.

12 with respect to the placebo group (with an OR = 4, $p = 0.0230$) (data not shown).

Finally, a biochemical evaluation of blood AD biomarkers was performed with measurements of tau aggregation in platelets [22] and AβPP in platelets [13], but we found no statistical differences between groups at the 24-week- follow-up.

DISCUSSION

The aim of the present study was to evaluate the actions of a 24-week treatment with BU-10 on apathy, cognition, and neuropsychiatric symptoms of AD patients in a placebo-controlled study. Besides the current observations of several years that patients treated with BU-10 in Chile exhibited a marker decrease in apathy, initial clinical evidence [7] indicated that apathy levels were an important aspect to be evaluated in this study. Apathy is the most commonly reported neuropsychiatric symptom in AD, and it has a very significative impact on quality of life and caregiver distress and may, or may not, be associated with depressive symptoms. Since apathy appears to potentiate functional and cognitive deterioration, it is a very important target for AD treatment [23]. AES is a widely used instrument for measurement of apathy in AD and other neurological conditions. Moreover, the clinician version of AES is an adequate instrument to predict conversion from mild cognitive impairment to AD [24] and to measure symptoms of apathy in AD subjects [25]. So, the tests AES I (informant test) and AES C (Clinician test) appear as reliable instruments to evaluate apathy and factors related to interest in life [26], although capacity of these tests to evaluate multidimensional characteristics of apathy has been somewhat questioned [27]

Here we show that treatment with BU-10 lowers apathy at two timepoints, i.e., week 4 and 12, as compared to placebo, although this effect did not reach a statistically significant level at other timepoints in AES. It is noteworthy the finding of a signi-

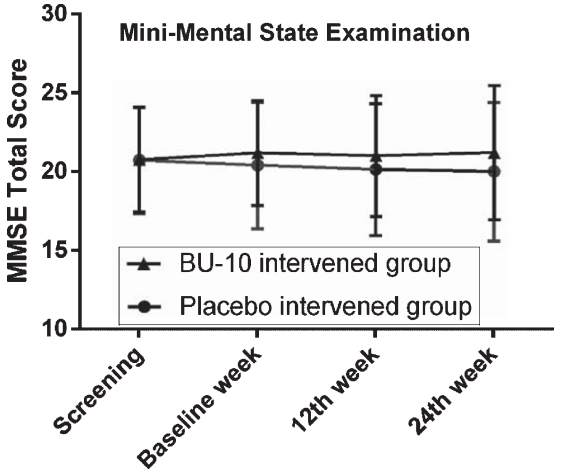

Fig. 4. The MMSE measures selected cognitive aspects such as memory, orientation, attention, language, and praxis on a scale of 0–30. MMSE was widely used to assess the overall cognitive status. Lower scores indicate greater cognitive decline. The MMSE was performed at the initial evaluation, week 12, and week 24. MMSE scores were comparison between groups during the 24 weeks. Although no statistically significant differences were found between the groups during the 24 weeks of the study, the BU-10 intervened group appears to maintain a more constant performance in relation to the decreased placebo intervened group.

ficant increase in the frequency of "interest in things" and "get things done during the day" for the BU-10 group as compared with placebo. Considering the impact of apathy in caregiver distress, it is also important to mention that the effect of BU-10 was most noticeable in the informant version of AES, i.e., with the interview of the caregiver, where significant differences were observed. It is possible that more pronounced neuropsychiatric, and even cognitive, effects may become more evident with a larger number of subjects and a more prolonged follow-up. This may also explain why there were no clear differences between groups in the apathy dominium of NPI, since this questionnaire is conceived for a wide evaluation of neuropsychiatric symptoms and does not explores apathy in detail, nor consider the multiple domains of the symptom. It is interesting that when we evaluated data from each domain of NPI, we found significant differences with lower scores in symptoms of delusion (Fig. 3A) and caregiver distress due to this symptom (Fig. 3B) as compared with the placebo data (Table 3).

In this study, we did not find significant differences in the ADAS-cog evaluation scale, or in relation to the level of blood biomarkers, both for tau and for APP in platelets; however, this may be related to the short follow-up period (24 weeks), since *in vitro* studies showed the effect of fulvic acid and BU-10 in reducing tau aggregation [28]. In turn, the platelet tau protein biomarker had a direct relationship with the clinical diagnosis of the study subjects.

Anyhow, considering the absence of effective treatments against apathy in neurodegenerative diseases and the relation between apathy and disease progression in AD [23], and the lack of effective therapies for neuropsychiatric distress in AD patients, studies suggest that BU-10 provides a solution that appears to impact beneficially in the functional status of patients, caregiver distress, and eventually disease progression in AD.

ACKNOWLEDGMENTS

We acknowledge the financial support of a grant from Corfo Innova # 14IEAT-28658, a FONDEF project ID19I10301 from ANID, the International Center for Biomedicine (ICC), and a grant from the "Fundación Ricardo Benjamin Maccioni". We also thank Constanza Maccioni for her outstanding dedication to the follow up and coordination of the study, and to Nicole Cortes for their invaluable support for the successful development of this clinical study.

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/20-1501r1).

REFERENCES

[1] Ishii M, Iadecola C (2020) Risk factor for Alzheimer's disease breaks the blood-brain barrier. *Nature* **581**, 31-32.

[2] Cortes N, Andrade V, Maccioni RB (2018) Behavioral and neuropsychiatric disorders in Alzheimer's disease. *J Alzheimers Dis* **63**, 899-910.

[3] Andrade V, Guzman-Martínez L, Cortes N, Maccioni RB (2019) The promise for Alzheimer disease treatment: Bioactive compounds In *Natural Medicines: Clinical Efficacy, Safety and Quality*, Dilip Ghosh, Mukherjee PK, eds. CRC Press.

[4] Vermeiren Y, Van Dam D, Aerts T, Engelborghs S, De Deyn PP (2014) Brain region-specific monoaminergic correlates of neuropsychiatric symptoms in Alzheimer's disease. *J Alzheimers Dis* **41**, 819-833.

[5] Steinberg M, Tschanz JT, Corcoran C, Steffens DC, Norton MC, Lyketsos CG, Breitner JC (2004) The persistence of neuropsychiatric symptoms in dementia: The Cache County Study. *Int J Geriatr Psychiatry* **19**, 19-26.

[6] Mega MS, Cummings JL, Fiorello T, Gornbein J (1996) The spectrum of behavioral changes in Alzheimer's disease. *Neurology* **46**, 130-135.

[7] Rog LA, Park LQ, Harvey DJ, Huang CJ, Mackin S, Farias ST (2014) The independent contributions of cognitive impairment and neuropsychiatric symptoms to everyday function in older adults. *Clin Neuropsychol* **28**, 215-236.

[8] van der Linde RM, Matthews FE, Dening T, Brayne C (2017) Patterns and persistence of behavioural and psycho-

logical symptoms in those with cognitive impairment: The importance of apathy. *Int J Geriatr Psychiatry* **32**, 306-315.

[9] Theleritis CG, Siarkos KT, Politis AM (2019) Unmet needs in pharmacological treatment of apathy in Alzheimer's disease: A systematic review. *Front Pharmacol* **10**, 1108.

[10] Carrasco-Gallardo C, Guzman L, Maccioni RB (2012) Shilajit: A natural phytocomplex with potential procognitive activity. *Int J Alzheimers Dis* **2012**, 674142.

[11] Carrasco-Gallardo C, Farias GA, Fuentes P, Crespo F, Maccioni RB (2012) Can nutraceuticals prevent Alzheimer's disease? Potential therapeutic role of a formulation containing shilajit and complex B vitamins. *Arch Med Res* **43**, 699-704.

[12] Neumann K, Farias G, Slachevsky A, Perez P, Maccioni RB (2011) Human platelets tau: A potential peripheral marker for Alzheimer's disease. *J Alzheimers Dis* **25**, 103-109.

[13] Borroni B, Colciaghi F, Pastorino L, Pettenati C, Cottini E, Rozzini L, Monastero R, Lenzi GL, Cattabeni F, Di Luca M, Padovani A (2001) Amyloid precursor protein in platelets of patients with Alzheimer disease: Effect of acetylcholinesterase inhibitor treatment. *Arch Neurol* **58**, 442-446.

[14] Marin RS, Biedrzycki RC, Firinciogullari S (1991) Reliability and validity of the Apathy Evaluation Scale. *Psychiatry Res* **38**, 143-162.

[15] Marin RS (1996) Apathy: Concept, syndrome, neural mechanisms, and treatment. *Semin Clin Neuropsychiatry* **1**, 304-314.

[16] Kueper JK, Speechley M, Montero-Odasso M (2018) The Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog): Modifications and responsiveness in pre-dementia populations. A narrative review. *J Alzheimers Dis* **63**, 423-444.

[17] Rabin LA, Barr WB, Burton LA (2005) Assessment practices of clinical neuropsychologists in the United States and Canada: A survey of INS, NAN, and APA Division 40 members. *Arch Clin Neuropsychol* **20**, 33-65.

[18] Reitan RM, Wolfson D (1995) Category test and trail making test as measures of frontal lobe functions. *Clin Neuropsychol* **9**, 50-56.

[19] Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**, 189-198.

[20] Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J (1994) The Neuropsychiatric Inventory: Comprehensive assessment of psychopathology in dementia. *Neurology* **44**, 2308-2314.

[21] Balestroni G, Bertolotti G (2012) [EuroQol-5D (EQ-5D): An instrument for measuring quality of life]. *Monaldi Arch Chest Dis* **78**, 155-159.

[22] Farias G, Perez P, Slachevsky A, Maccioni RB (2012) Platelet tau pattern correlates with cognitive status in Alzheimer's disease. *J Alzheimers Dis* **31**, 65-69.

[23] Nobis L, Husain M (2018) Apathy in Alzheimer's disease. *Curr Opin Behav Sci* **22**, 7-13.

[24] Guercio BJ, Donovan NJ, Munro CE, Aghjayan SL, Wigman SE, Locascio JJ, Amariglio RE, Rentz DM, Johnson KA, Sperling RA, Marshall GA (2015) The Apathy Evaluation Scale: A comparison of subject, informant, and clinician report in cognitively normal elderly and mild cognitive impairment. *J Alzheimers Dis* **47**, 421-432.

[25] Hsieh CJ, Chu H, Cheng JJ, Shen WW, Lin CC (2012) Validation of apathy evaluation scale and assessment of severity of apathy in Alzheimer's disease. *Psychiatry Clin Neurosci* **66**, 227-234.

[26] Radakovic R, Harley C, Abrahams S, Starr JM (2015) A systematic review of the validity and reliability of apathy scales in neurodegenerative conditions. *Int Psychogeriatr* **27**, 903-923.

[27] Radakovic R, Abrahams S (2014) Developing a new apathy measurement scale: Dimensional Apathy Scale. *Psychiatry Res* **219**, 658-663.

[28] Cornejo A, Jimenez JM, Caballero L, Melo F, Maccioni RB (2011) Fulvic acid inhibits aggregation and promotes disassembly of tau fibrils associated with Alzheimer's disease. *J Alzheimers Dis* **27**, 143-153.