FARMACI

AGGIORNAMENTO CONTINUO PER LA PRATICA CLINICA

©2020 • Volume 19 • N. 2 (Estratto)

Direttore Responsabile: Antonio Guastella - ©2020 MEDIZIONI S.r.l. - Cod. 16/20 - Via Monte delle Gioie, 13 - 00199 Roma - Tel. 06.81153040/06.40413168 Fax. 06.40419131 - medizioni@medizioni.it - medizioni@ohtpec.it - Reg. Trib. di Roma n. 238 del 23/5/2002 - Periodicità quadrimestrale - Tutti i diritti sono riservati. Nessuna parte può essere riprodotta in alcun modo (comprese fotocopie), senza il permesso scritto dell'editore. Stampa: CSC Grafica Srl - Via A. Meucci, 28 - 00012 Guidonia (Roma) - Estratto finito di stampare nel mese di settembre 2020

Direttore Scientifico: **Ercole Concia** - Direttore **Editoriale: Matteo Bassetti**

Prospective comparative pilot study to evaluate the efficacy of a compound containing L-acetylcarnitine, *Ginkgo biloba* extract and vitamin B12 in the treatment of patients diagnosed with vestibular neuritis

Paolo Marras[1], Stefano Agostini[2]

[1]Surgeon Specialist in Otorinolaryngology, Qualified to Treat Patients with Obstructive Sleep Apnoea, University Master's Degree in Practical Vestibology, Free Professional, Cagliari;

[2]Pharma Line, Milan

Abstract

Vestibular neuritis (VN), also referred to as vestibular neuronitis or acute vestibular deficit in the scientific literature, is defined as sudden loss of function in one peripheral vestibular apparatus or, more rarely, in both of them.

Vestibular rehabilitation is the treatment of choice for most patients with VN.

It has been shown to be effective, leading to changes in the central nervous system and to the development of new sensory and behavioural strategies defined as vestibular compensation.

The purpose of this pilot study is to investigate the therapeutic possibilities of an oral compound containing L-acetylcarnitine, Ginkgo biloba extract, vitamin B12 associated with

vestibular rehabilitation in the treatment of patients with VN. In particular, this study aims to assess whether the combination of orally administered Brainil®, a nutraceutical product containing such ingredients, and vestibular rehabilitation can increase therapeutic effect better than vestibular rehabilitation alone.
This prospective comparative pilot study was conducted in 40 patients (12 males, 28 females; mean age 57.5 years, minimum age 27, maximum age 79) with VN.
Patients were divided into two groups of 20 patients: the Brainil group (7 males, 13 females; mean age 56 years, minimum age 27 years, maximum age 79 years) and the control group (5 males, 15 females; mean age 59 years, minimum age 47 years, maximum age 71 years).
Patients in the Brainil group underwent vestibular rehabilitation combined with the administration of oral Brainil at the dose of 1 tablet for 90 consecutive days, while patients in the control group only underwent vestibular rehabilitation.
Our results show that patients undergoing oral treatment with Brainil combined with vestibular rehabilitation physical therapy have significant improvement in quantitative recovery of vestibulo-oculomotor reflex and in the normalization of stabilometric parameters (index of cervical interference) as well as a tendency for better results in the other considered parameters, compared to vestibular rehabilitation physical therapy alone.
Treatment with Brainil did not induce any adverse effect and no patient discontinued treatment, confirming its high tolerability.

Introduction

Vestibular neuritis (VN), also referred to as vestibular neuronitis or acute vestibular deficit in the scientific literature, is defined as sudden loss of function in one peripheral vestibular apparatus or, more rarely, in both of them. Correct differential diagnosis is essential because similar clinical pictures can have different, sometimes very serious, etiologies.
Anatomopathological examination of the temporal bone of patients with this disease shows that VN can have viral or ischaemic origin. Degenerative lesions in the portions of the vestibular nerve, with variable involvement of the receptor neuroepithelium, are usually detected, constituting the most important pathological feature of this disease. VN is characterized by a lesion in the upper portion of the vestibular labyrinth (utricle and lateral and anterior semicircular channels) which is innervated by the superior division of the vestibular nerve, while the lower portion of the labyrinth (saccule and posterior semicircular channel) is generally spared.
An episode of influenza or herpes before onset of VN (1-5) is generally reported, supporting a viral origin with subsequent inflammatory lesion (1-5). The upper vestibular nerve is mostly affected because its fibers are longer than those of the lower vestibular nerve.
In patients with clear cardiovascular risk factors (hypertension, obesity, hypercholesterolemia, diabetes, cigarette smoking, a history of cerebrovascular ischemic attacks) VN of ischemic origin has been suspected, because of the fact that the inner ear is supplied by a terminal artery (6). The territory of the upper vestibular artery, which is also innervated by the upper vestibular nerve, i.e. the utriculus and the lateral and anterior semicircular channels, is particularly vulnerable to ischaemia.
The epidemiological incidence of VN cannot be established with certainty as few studies have been properly conducted. In a study conducted in Japan, the incidence of this disease was approximately 3,5 new cases per 100.000 inhabitants (7), but this datum is likely to be underestimated. In fact, in a study conducted in Europe, the annual incidence ranged between 11.7 and 15.5 cases per 100.000 (8). Patients are usually aged between 30 and 60 years; men and women are equally affected.
Clinical status is characterized by the onset of rotatory ver-

tigo associated with static or dynamic postural instability sometimes accompanied by neurovegetative symptoms such as nausea, vomiting and profuse sweating which continue for 1-3 days. Clinical examination shows spontaneous nystagmus, pathological cephalic impulse test (Halmagyi-Curthoys Head Impulse Test) and a tendency to fall towards the side of the affected ear. Bithermal stimulation shows unilateral vestibular hypofunction or unilateral complete loss of vestibular function. Acute phase covers a period of two weeks (9), followed by subacute phase, which can last up to three months. If typical signs and symptoms persist, the disease is considered chronic. Persistent symptoms lasting for months or years have been reported in 30-50% of patients (10-12).

Vestibular rehabilitation is designed to reduce dizziness and improve balance as well as general physical function (9,13). The relationship with patient is a key element in vestibular rehabilitation, aiming to reduce anxiety and to promote a better understanding of the underlying disease. Vestibular rehabilitation includes measures intended to reduce susceptibility to movement (habituation exercises) as well as to improve gaze stability (head-eye movement exercises), balance and to increase endurance (9). Vestibular rehabilitation is the treatment of choice for the majority of patients with VN. It has been shown to be effective, leading to changes in the central nervous system and to the development of new sensory and behavioural (14,15) strategies defined as vestibular compensation (16). Evidence from randomized controlled clinical trials involving patients with VN highlights the importance of early vestibular rehabilitation (17-20). A recent randomized, controlled clinical trial confirmed the core role of vestibular rehabilitation therapy program immediately after confirmation of diagnosis (21). This study highlighted that the association between this program and standard drug therapy resulted in reduced perception of vertigo and in improved functions in real-life situations. These outcomes were significantly higher than those achieved with standard therapy alone (21).

Purpose of the study

The purpose of this pilot study is to investigate the therapeutic possibilities of an oral compound containing L-acetylcarnitine, *Ginkgo biloba* extract, vitamin B12 associated with vestibular rehabilitation in the treatment of patients with VN. In particular, this study aims to assess whether the association between orally administered nutraceutical product containing such ingredients and vestibular rehabilitation can increase therapeutic effects better than vestibular rehabilitation alone.

Materials and Methods

Tested dietary supplement

Brainil® (Pharma Line S.r.l. Milan, marketed since October 2004) is a dietary supplement in tablet form containing a mixture of L-acetylcarnitine, Ginkgoselect® Plus Fitosoma® (standardized *Ginkgo biloba* extract, titrated 7% ginkgo flavonlycosides and 0,8% bilobalide) and vitamin B12. Dietary supplement composition is shown in Table I.

Table I. *Qualitative and quantitative composition of Brainil®.*

Constituent	Content per tablet
L-acetylcarnitine (LAC) HCl containing L-acetylcarnitine equal to L-carnitine	590 mg 500 mg 396.5 mg
Ginkgo biloba, extract	80 mg
Vitamin B12	16.5 µg

Assessed population

The study enrolled forty patients (12 males, 28 females; mean age 57.5 years, minimum age 27, maximum age 79) with VN. Patients were randomly divided into two groups of 20 patients: the Brainil group (7 males, 13 females; mean age 56 years, minimum age 27 years, maximum age 79 years) and the control group (5 males, 15 females; mean age 59 years, minimum age 47 years, maximum age 71 years). The demographic characteristics and cardiovascular risk factors of enrolled patients are described in Table II.

The following exclusion criteria were applied: a history of chronic vestibular dysfunction; acute hearing loss; syntoms of a central nervous system injury; severe hypertension (systolic pressure >180 mm Hg or diastolic pressure >110 mm Hg); severe diabetes mellitus (blood glucose >180 mg/dL

Table II. *At therapy initiation, the treatment groups were homogeneous with regard to demographic characteristics and clinical profile. Cardiovascular (CV) risk factors included: overweight (n = 5), hypertension (n = 15), diabetes (n = 4), hypercholesterolaemia (n = 2). Six subjects had multiple risk factors (2 in the Brainil group, 4 in the control group).*

Demographic characteristics	Brainil (n = 20)	Control (n = 2 0)	p-value
Age in years, mean+SD	56 ± 13.4	59 ± 8.5	0.440
Gender, % F (n)	65 (13)	75 (15)	0.730
Lifestyle, % (n)			
Sedentary lifestyle	60 (12)	75 (15)	0.500
Sporty lifestyle	40 (8)	25 (5)	
Smoking, % YES (n)	25 (5)	25 (5)	1.000
Clinical profile			
CV risk, % YES (n)	35 (7)	50 (10)	0.522

despite appropriate pharmacological treatment); mental disorders; pregnant and breastfeeding women. Moreover, all patients with hypersensitivity to one or more of the ingredients in Brainil® were excluded, while particular attention was given to patients taking anticoagulants or antiplatelet drugs and undergoing polydrug treatment. Table III shows patients' existing drug regimen at enrollment.
During this process, all patients were duly informed on treatment protocol and signed the informed consent.

Table III. *Patients' existing drug regimen at enrollment. Some subjects took more than one drug at a time.*

Drug	Brainil (n)	Control (n)
Alpha-lithic	2	1
Beta-blocker	6	7
Nicardipine	1	2
Esomeprazole/omeprazole	5	4
Hypoglycemic agent	0	2
Statin	1	1
Fluoxetine	1	1
Estroprogestin pill	2	1

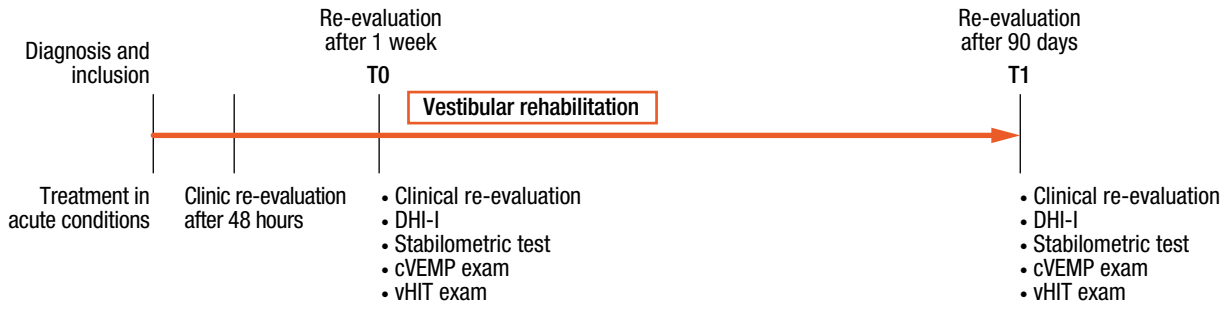
Study design

We conducted a prospective, comparative pilot study of two groups of patients with VN with the same numerical consistency.
Patients were enrolled and treated in the calendar year 2019, from 7 January 2019 to 20 December 2019.
We initially accurately recorded the medical history of patients with VN (object of study), including symptoms, predisposing factors and triggers as well as possible drug intake, previous diseases, cardiovascular risk factors and lifestyle. Patients' history was followed by otorhinolaryngoiatric examination and clinical test of vestibular function (bed side examination) using infrared videonystagmoscopy and complemented by audiologic instrumental evaluation (tone audiometry test and impedance audiometry with measurement of acoustic-stapedial reflexes). The diagnosis of VN, with suspected viral or embolic-vascular origin, was based on this evaluation scheme.
Patients immediately received drug therapy based on corticosteroids, nootropics, antithrombotic agents derived from heparin, calcium-channel blockers and antihistamines alone or in combination. Clinical follow-up of patients was conducted 48 hours after first assessment and initiation of therapy. Patients were reassessed after one week and this clinical re-evaluation was complemented with stabilometric test, Cervical Vestibular Evoked Myogenic Potential (cVEMP) testing, Video Head Impulse Test (vHIT) and the compilation of the Dizziness Handicap Inventory (DHI) Questionnaire – Italian Adaptation (DHI-I).
Time 0 (T0) corresponded to first data collection (Fig. 1). Patients started vestibular rehabilitation program the following day; they were divided into two groups:

- the Brainil group, consisting of 20 patients treated with vestibular rehabilitation associated with oral administration of Brainil® at the dose of 1 tablet between meals for 90 consecutive days;
- the control group consisted of 20 patients treated with vestibular rehabilitation alone.

Follow-up evaluations were scheduled 90 days after treatment and patients underwent all tests again. Time 1 (T1) corresponded to this second-stage data collection (Fig. 1).

Figure 1. *Study design and temporal division of the activities.*



Stabilometric test

It allows an instrumental evaluation of the postural stability of a motionless subject in an upright position based on multiparametric assessment of postural oscillations. Moreover, it analyzes the strategy used by the patient to maintain balance and postural stability and highlights the role of the different components of the postural system on the basis of modifications involving basal condition, using visual and proprioceptive stimulations. The vertical force platform (size 50 x 50 x 7 cm), equipped with 3 transducers placed at the vertices of an equilateral triangle measuring 400 mm on the side, is included in the based system of SVEP steady-state platform and provides the recording of patient's center of pressure movements (CoP). Parameters used are: the x-coordinate and the y-coordinate of CoP; the period of oscillation; the mean velocity and standard deviation of the displacements.

Parameters are recorded first with the patient's eyes open, then with the patient's eyes closed.

The percentage ratio between closed-eye and open-eye values for each parameter is known as Romberg's Index (RI). This index is normal when there is an increase in oscillating motions with eyes closed, with a value greater than 100. These parameters are also recorded with patient's head retroflected, both with open eyes and with closed eyes; head retroflexion allows to calculate the index of cervical interference (CI) which is normal with a value lower than 120.

The enrolled patients with VN, evaluated during the acute phase, showed pathological values of Romberg's Index (RI <100) and of the index of cervical interference (CI >120).

Cervical Vestibular Evoked Myogenic Potential (cVEMP)

It consists of recording of the electric potentials of the sternocleidomastoid muscles, generated following a mechanical-vibratory stimulation on the skull or intense acoustic stimulation. The normal tracking criteria included: the presence of Premature Biphasic Complex (PBC), normal morphology of the right and left curves, normal latency of the right and left curves, normal amplitude of the right and left curves. The patients with VN evaluated during the acute phase showed absence of PBC or the presence of pathologically reduced PBC amplitude and latency in 25% of cases.

Video Head Impulse Test (vHIT)

It is a clinical evaluation of the integrity of the vestibulo-oculomotor reflex (VOR) at high frequencies. The examination consists in a passive unpredictable high-acceleration head rotation imparted to the patient. Under normal conditions, eye movements are symmetrical during impulsive movements, responses are mediated by a single labyrinth, VOR gain (ratio between head movement and eye movement) is 1 and there are no eye movements bringing gaze back to the target (catch-up saccade).

The evaluated parameters are: VOR gain (normal if higher than 0.75), absence or presence of catch-up saccade (normal if saccades are absent), characterization of any catch-up saccade (overt, covert). Patients with VN, evaluated during

the acute phase, showed pathological values of VOR gain, the presence of overt catch-up saccade (reported after head rotation, perceptible to the examiner) or covert catch-up saccade (described during head rotation, not perceptible to the examiner) or both.

Dizziness Handicap Inventory Questionnaire – Italian Adaptation (DHI-I)

The questionnaire is a structured interview on physical, emotional and functional problems associated with balance disorders. It allows to quantify the subjective perception of disability of a patient suffering from balance disorders based on dizzy patients' difficulties in describing symptoms and their intensity and considering that the concept of vertigo can be variously interpreted.
The DHI questionnaire comprises 25 questions divided into 3 domains: 9 emotional, 9 functional and 7 physical questions. Each answer is given a score: (0) NO, (2) SOMETIMES, (4) YES. A total score of 0 to 30 is rated as absence of disability or mild disability; 31 to 60 as average disability; 61 to 100 as high disability or total disability. A score improvement of at least 10% is clinically significant.
Patients received the questionnaire at initiation of the otovestibular evaluation in the clinic. They responded to the questions autonomously without being conditioned by the physician.
At initiation of their respective treatments (T0) both groups of patients were homogeneous for the specific parameters investigated in the study (Tab. IV).

Table IV. *At initiation of their respective treatments (T0) both groups of patients were homogeneous for the specific parameters investigated in the study.*

Parameter	Brainil	Control	p-value
DHI-I, mean ± SD	48.5 ± 17.2	51.5 ± 19.3	0.388
Stabilometry, median (min-max)			
RI	83.5 (74-133)	85 (69-174)	0.892
CI	99 (77-185)	105.5 (77-188)	0.685
Pathological cVEMP, % (n)	50 (10)	50 (10)	1.000
vHIT			
VOR gain, mean ± SD	61.7 ± 6.1	61.8 ± 6.6	0.625
Saccades, % (n)	90 (18)	90 (18)	1.000

Vestibular rehabilitation

Rehabilitation techniques were designed to the re-education of ocular motricity, postural control and control of locomotion. The exercises were performed in the clinic with the help of a professional (audiometrist, physiotherapist) on a weekly basis for the first month, on a biweekly basis for the second month. The last session was conducted after a further month. Passive exercises were carried out; they lasted for about 20 minutes and then patients independently performed them at home, at least once a day.

Statistical analysis

Descriptive statistics was used to summarize the features of patient cohort in terms of mean and standard deviation, median (min; max) or frequencies, when deemed appropriate.
The differences between basic conditions in the two treatment groups were assessed by the t-test or nonparametric test for continuous variables and by Fisher's exact test assessing frequencies.
The effect of treatment was evaluated in terms of variation in the results between the T1 visit and the T0 visit. The meaningfulness of the differences was determined by the non-parametric Mann-Whitney test with paired data for the comparision between T1 and T0 and with non-paired data for the assessment of changes between the two treatment groups.
In all performed analyses the results were considered statistically significant for $p < 0.05$.
R version 3.6.1 software for Windows was used for the statistical elaboration (R Core Team; 2013. A language and environment for statistical computing. R Foundation for Statisti-cal Computing, Vienna, Austria).

Results

Stabilometric test

Treatments of patients in the Brainil group and in the control group were proven effective in improving the measured RI value. At the end of the 90 days of treatment (T1), the increase in the RI value was statistically significant both in the Brainil group (median increase: + 24; $p < 0.001$; Fig. 2A) and in the control group (median increase: + 10; $p = 0.003$; Fig. 2B). A comparision of increases in the RI value between the Brainil group and the control group found a more marked improve-

Figure 2. *The increase in the RI value was statistically significant in both the Brainil group (A) and the control group (B). A more marked improvement can be observed in the Brainil group compared to the control group (C). In the graphs, each dot represents the value for a single patient, the orange line indicates the median.*

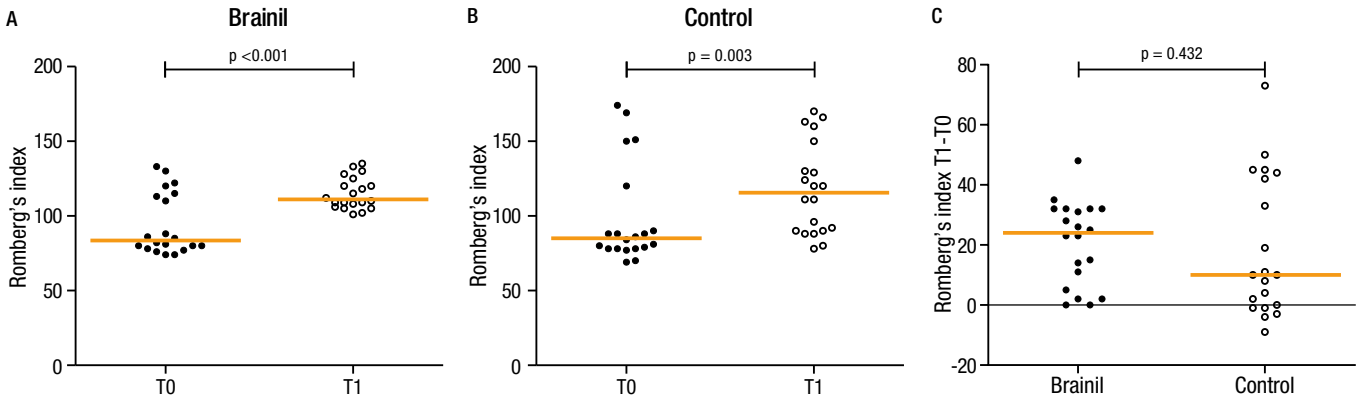


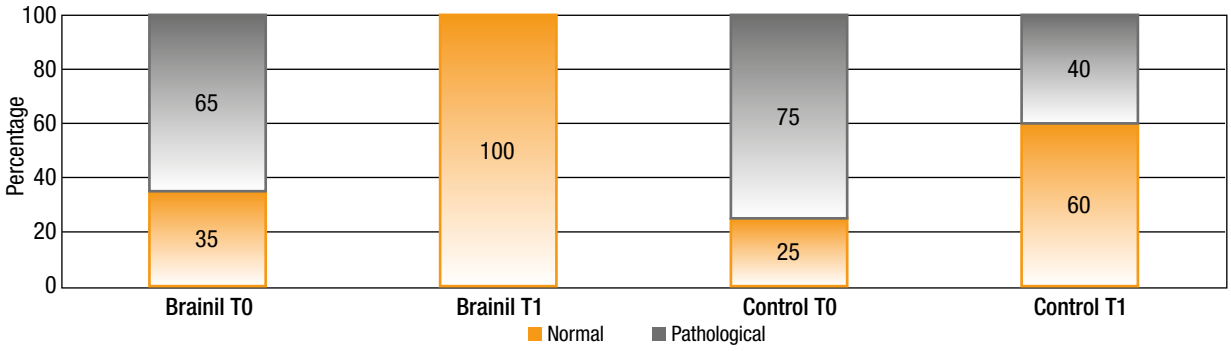
Table V. *Medians of RI value measurements collected in the Brainil group and in the control group at T0 and T1. A comparision of increases in the RI value between the Brainil group and the control group found a more marked improvement in the Brainil group compared to the control group.*

Group	T0 (median; min-max)	T1 (median; min-max)	T1-T0 (median; min-max)
Brainil	83.5 (74.0-133.0)	111.0 (101.0-135.0)	24.0 (0.0-48.0)
Control	85.0 (69.0-174.0)	115.5 (78.0-170.0)	10.0 (-9.0-73.0)

ment in the Brainil group compared to the control group (p = 0.432; Tab. V; Fig. 2C).

Calculating the difference between the percentage of subjects with normal values at T1 versus T0 (% at T1 - % at T0), it emerged that at the end of the 90 days of treatment (T1), 65% of subjects in the Brainil group and 35% of subjects in the control group changed from pathological RI values at T0 to normal values at T1. The difference between the two treatment groups, as regards the percentages of patients whose values returned to normal during the 90 days of treatment, was therefore equal to 30% and was at the limit of statistical significance (p = 0.055; Fig. 3). At T1 all patients

Figure 3. *Percentages of patients with pathological or normal RI values at T0 and T1. The difference between the percentages of patients in the two groups whose parameters returned to normal after 90 days of treatment was 30% in favor of the Brainil group and was at the limit of statistical significance (p = 0.055).*



treated with Brainil® had normal RI values, while 40% of patients in the control group still showed pathological RI values. The treatment of patients in the Brainil group was proven effective in reducing the measured CI value. On the contrary, the treatment of patients in the control group wasn't proven effective in reducing the measured CI value. In fact, at the end of treatment (T1), there was a statistically significant reduction in the CI value in the Brainil group (p = 0.024; Fig. 4A) but this was not significant in the control group (p = 0.076; Fig. 4B). A comparision of reductions in the CI value between

Figure 4. *The reduction in the CI value was statistically significant in the Brainil group (A), while it was not significant in the control group (B). In the graphs, each dot represents the value for a single patient, the orange line indicates the median.*

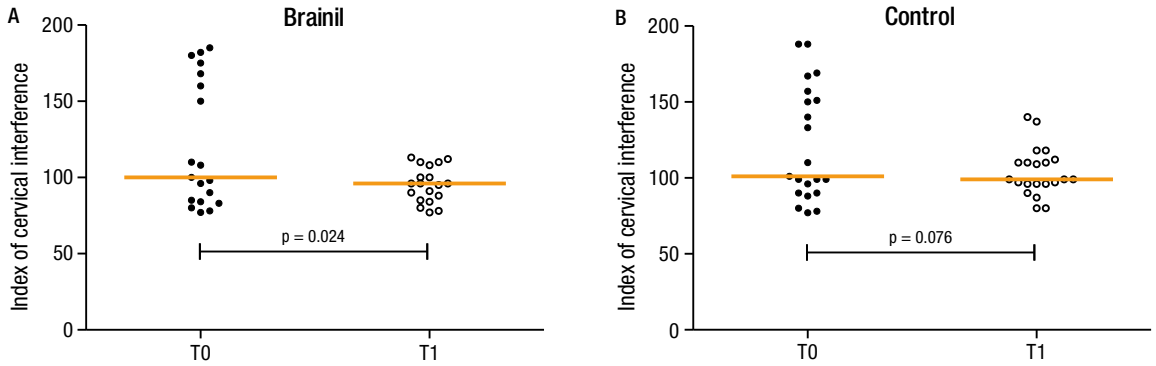
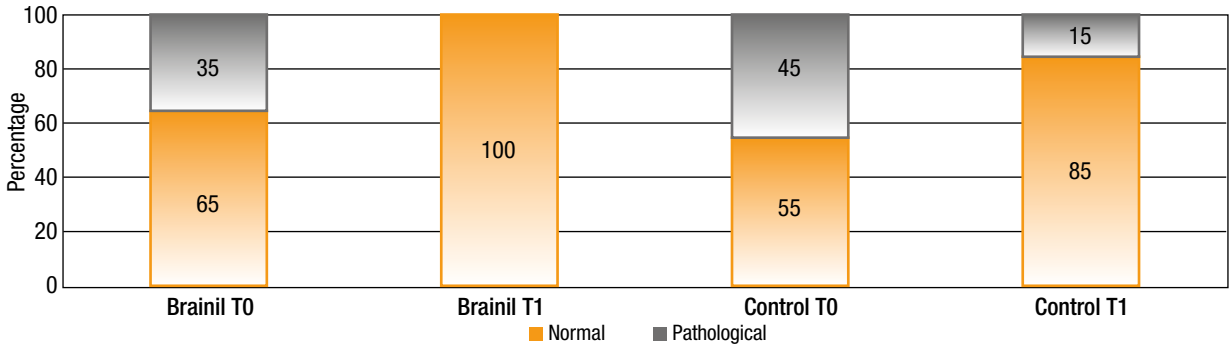


Table VI. *Medians of CI value measurements collected in the Brainil group and in the control group at T0 and T1. A comparision of reductions in the CI value between the Brainil group and the control group found a more marked reduction in the Brainil group compared to the control group.*

Group	T0 (median; min-max)	T1 (median; min-max)	T1-T0 (median; min-max)
Brainil	99.0 (77.0-185.0)	96.0 (77.0-113.0)	-3.5 (-85.0-29.0)
Control	105.5 (77.0-188.0)	99.0 (80.0-140.0)	-3.0 (-78.0-29.0)

Figure 5. *Percentages of patients with pathological or normal CI values at T0 and T1. The difference between the percentages of patients in the two groups whose values returned to normal after 90 days of treatment was 5% in favor of the Brainil group (p = 0.377).*



the Brainil group and the control group found a more marked improvement in the Brainil group compared to the control group, justifying the statistically significant difference detected (Tab. VI).

Calculating the difference between the percentage of subjects with normal values at T1 versus T0 (% at T1 -% at T0), it emerged that at the end of the 90 days of treatment (T1), 35% of subjects in the Brainil group and 30% of subjects in the control group changed from pathological CI values at T0 to normal values at T1. The difference between the two treatment groups, as regards the percentages of patients whose values returned to normal during the 90 days of treatment, was therefore equal to 5% (p = 0.377; Fig. 5). At T1 all patients treated with Brainil had normal CI values, while 15% of patients in the control group still showed pathological CI values.

Cervical Vestibular Evoked Myogenic Potential (cVEMP)

The treatments of patients in the Brainil group and in the control group were proven effective in improving the cVEMP test result (Brainil group: p = 0.004; control group: p = 0.041). Calculating the difference between the percentage of subjects with normal values at T1 versus T0 (% at T1 -% at T0), it emerged that at the end of the 90 days of treatment (T1) 50% of subjects in the Brainil group and 30% of subjects in the control group-changed from pathological condition at T0 (evaluated by means of cVEMP) to normal values at T1. The difference between the two treatment groups, as regards the percentages of patients whose values returned to normal during the 90 days of treatment, was therefore equal to 20% (p = 0.167; Fig. 6). At T1 all patients treated with Brainil® had normal values, while 20% of patients in the control group still showed pathological values.

Video Head Impulse Test (vHIT)

The treatments of patients in the Brainil group and in the control group were proven effective in improving the VOR gain. In fact, at the end of the 90 days of treatment (T1), the increase in VOR gain compared to T0 was statistically significant both in the Brainil group (p <0.0001; Fig. 7A) and in the control group (p <0.0001; Fig. 7B). A comparision of increases in VOR gain between the Brainil group and the control group found a more marked improvement in the Brainil group compared to the control group; this difference was statistically significant (p = 0.005; Tab. VII; Fig. 7C)

At T0, 100% of patients in the two treatment groups had pathological VOR values. At the end of the 90 days of treatment (T1), 80% of subjects in the Brainil group and 70% of subjects in the control group changed from pathological VOR values at T0 to normal values at T1. The difference between the two treatment groups as regards the percentages of patients whose values returned to normal during the 90 days of treatment was therefore 10% (p = 0.305; Fig. 8).

At T0, 90% of patients in the Brainil group and in the control group had saccades. Calculating the difference betwe-

Figure 6. *Percentage of patients with pathological or normal clinical findings at T0 and T1 according to cVEMP assessment. The difference between the percentages of patients in the two groups whose values returned to normal after 90 days of treatment was 20% in favor of the Brainil group (p = 0.167).*

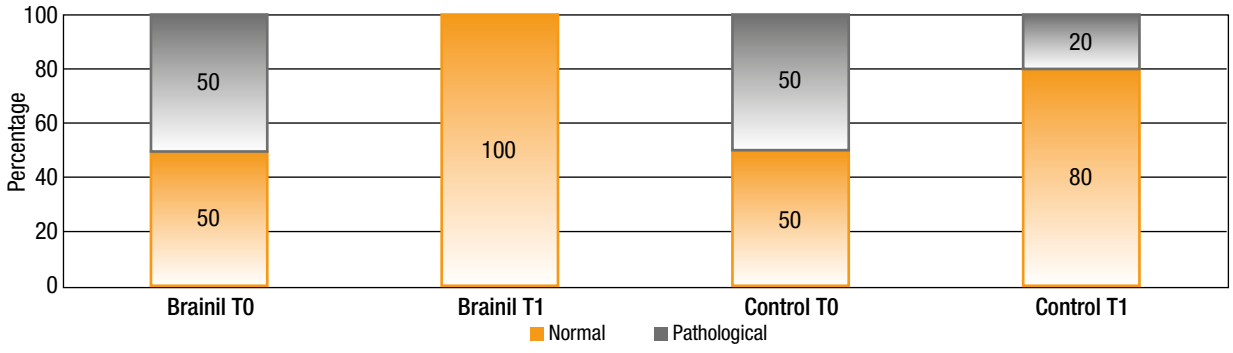


Figure 7. *The increase in the VOR gain was statistically significant in both the Brainil group (A) and the control group (B). A more marked improvement was observed in the Brainil group compared to the control group. This difference was statistically significant (C). In the graphs, each dot represents the value for a single patient, the orange lines indicate the mean ± standard error.*

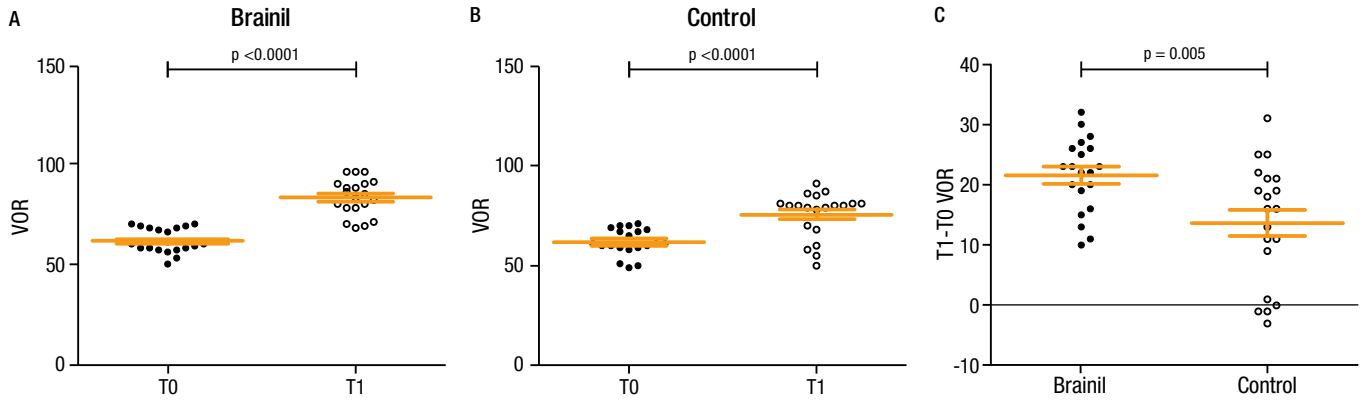
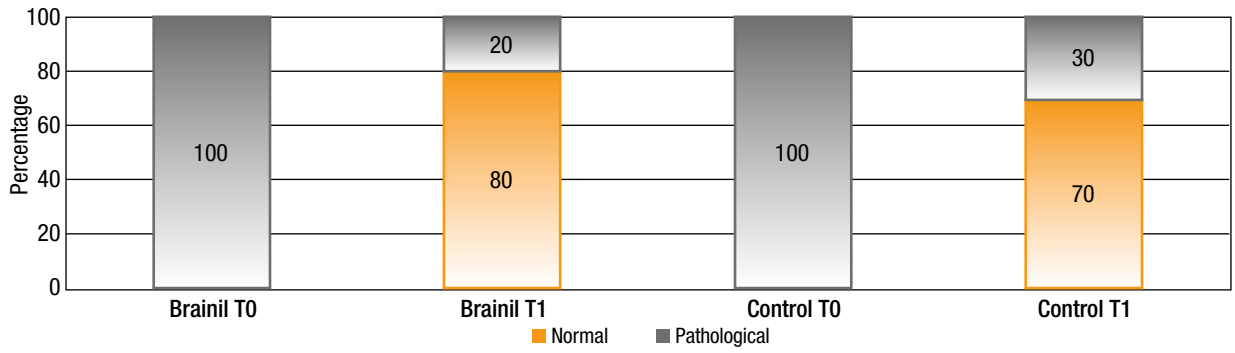


Table VII. *Means of VOR gain measurements collected in the Brainil group and in the control group at T0 and T1. A comparision of increases in VOR gain between the Brainil group and the control group found a more marked improvement in the Brainil group compared to the control group. The difference between these two groups was statistically significant (p = 0.005).*

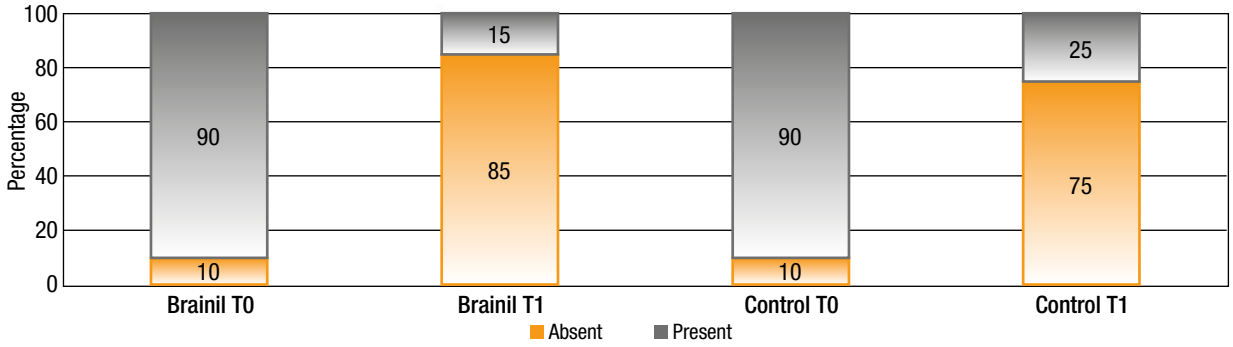
Group	T0 (mean+SD)	T1 (mean+SD)	T1-T0 (mean+SD)
Brainil	61.7 ± 6.1	83.3 ± 9.0	21.6 ± 6.1
Control	61.8 ± 6.6	75.5 ± 11.4	13.7 ± 10.0

Figure 8. *Percentage of patients with pathological or normal clinical findings at T0 and T1 determined on the basis of VOR gain assessment. The difference between the percentages of patients in the two groups whose values returned to normal after 90 days of treatment was 10% in favor of the Brainil group (p = 0.305).*



en the percentage of subjects without saccades at T1 versus T0 (% at T1 -% at T0), it emerged that at the end of the 90 days of treatment (T1), saccade cancellation involved 75% of subjects in the Brainil group and 65% of subjects in the control group. The difference between the two treatment groups, as regards the percentages of patients whose parameters returned to normal during the 90 days of treatment, was therefore equal to 10% (p = 0.314; Fig. 9). At

Figure 9. *Percentages of patients with or without saccades at T0 and T1. The difference between the percentages of patients in the two groups whose values returned to normal after 90 days of treatment was 10% in favor of the Brainil group (p = 0.314).*



T1 saccades were absent in 85% of patients in the Brainil group and in 75% of patients in the control group.

Dizziness Handicap Inventory Questionnaire – Italian Adaptation (DHI-I)

The treatments of patients in the Brainil group and in the control group were proven effective in reducing the score of the DHI-I questionnaire and dysability perceived by patients. In fact, from T0 to T1, a statistically significant reduction in the score of the DHI-I questionnaire both in the Brainil group (p <0.0001; Fig. 10A) and in the control group (p <0.0001; Fig. 10B) was observed. A comparision

Figure 10. *The reductions in the scores of the DHI-I questionnaire were statistically significant in both the Brainil group (A) and the control group (B). There was no difference between the two groups (C). In the graphs, each dot represents the value for a single patient, the orange lines indicate the mean ± standard error.*

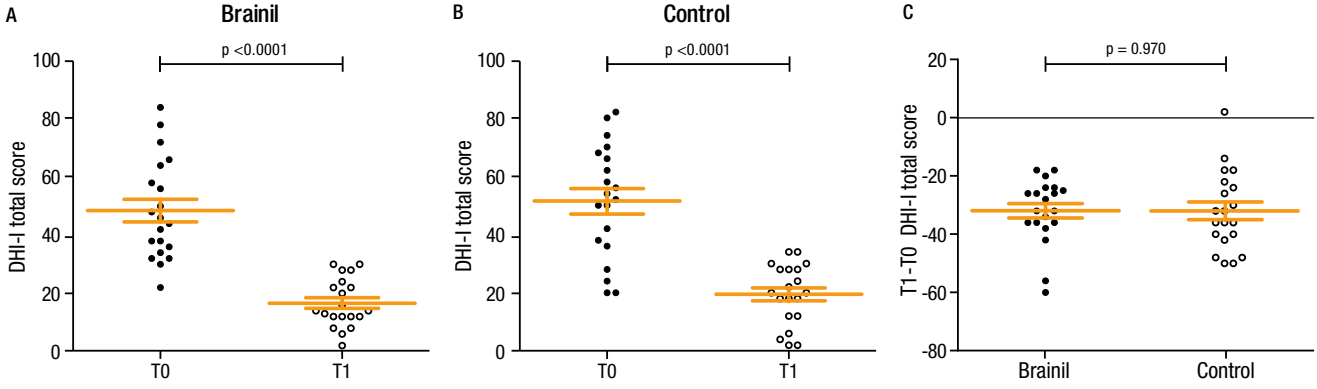
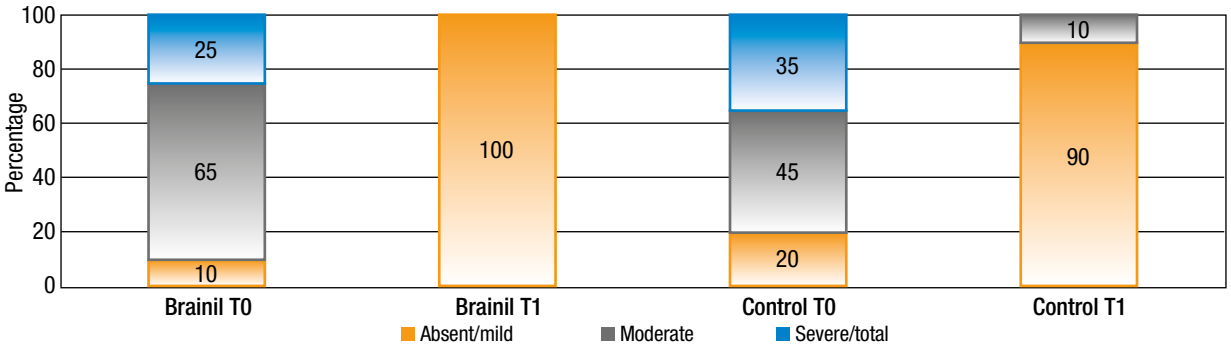


Table VIII. *Means of the scores of the DHI-I questionnaire in the Brainil group and in the control group at T0 and T1. A comparision of reductions in the scores between the Brainil group and the control group found no difference (p = 0.970).*

Group	T0 (mean+SD)	T1 (mean+SD)	T1-T0 (mean+SD)
Brainil	48.5 ± 17.2	16.7 ± 8.4	-31.9 ± 11.2
Control	51.5 ± 19.3	19.5 ± 10.4	-32.0 ± 13.6

Figure 11. *Percentages of patients experiencing severe or total disability, moderate disability and mild disability or absence of disability at T0 and T1. These were assessed using the DHI-I questionnaire. The difference between the percentages of patients who experienced a reduction in perceived disability in the two treatment groups was 10% in favor of the Brainil group (p = 0.268).*



of reductions in the questionnaire score between the Brainil group and the control group found no significant difference (p = 0.970; Tab. VIII; Fig. 10C).

Calculating the difference between the percentage of subjects who experienced a reduction in perceived disability at T1 versus T0 (% at T1 -% at T0) using the DHI-I questionnaire, it emerged that at the end of the 90 days of treatment (T1), 90% of subjects in the Brainil group and 80% of subjects in the control group experienced a reduction in perceived disability. The difference between the two treatment groups, as regards the percentages of patients experiencing a reduction in perceived disability during the 90 days of treatment, was therefore equal to 10% (p = 0.268; Fig. 11). At T1, 100% of patients treated with Brainil didn't perceive their disability or perceived mild disability, while in the control group, 10% of patients still perceived moderate disability.

Adverse effects

During the whole treatment period, no patient showed treatment-related adverse effects and no patient discontinued therapy.

Discussion

During the acute phase of vestibular injury, the predominant clinical symptom is vertigo. Early and accurate diagnosis and therapy are fundamental; otherwise, at a later stage, imbalance persists in predisposed subjects and its chronicization manifests as new patient's perception of the surrounding space. This can lead to an alteration of body self-awareness due to erroneous perception of one's own position in space, of movement and position of memorized objects and to erroneous calculation of the linear and angular distances. In Italy, epidemiological incidence of vestibular injuries is about 5,5% (about 3.300.000 people), representing the first reason for ENT consultation among patients aged more than 75 years (22).

Vestibular lesions require early and accurate diagnosis and treatment. VN is defined as sudden loss of function in one peripheral vestibular apparatus or, more rarely, in both of them. It is one of the most common peripheral vestibular disorders.

At the present time, there is no single, established and univocally acknowledged drug therapy for VN, while physical rehabilitative therapy is an indispensable support for patients in the recovery of vestibular functional integrity.

Ideally, vestibular pharmacotherapy is designed to significantly relieve symptoms of vertigo, to protect or repair vestibular sensory network altered by patient's pathological condition and to enhance vestibular compensation using specific and targeted molecular actions. Its ultimate purpose is to improve patient's quality of life.

In the field of conventional pharmacology, significant progresses are needed to achieve this goal. The lack of information about the etiology of vestibular disorders and the

pharmacological targets to modulate, as well as the technical challenge of targeting drug to the most appropriate site, are some of the fundamental problems to overcome (23).

An interesting opportunity is provided by nutraceuticals combining therapeutic actions which can be contextualized for use within clinical treatment of VN with significant safety, which makes them helpful for long-term therapies. This is also the reason why nutraceuticals are generally appreciated by patients.

The etiology of VN has not yet been definitively ascertained, but it is assumed to be caused by an infection/inflammation in patients with viral infections or by ischemia in patients with cardiovascular risk factors. Based on these assumptions, a nutraceutical product suitable for the treatment of VN should contain ingredients targeted to the nerves and blood vessels with anti-inflammatory, antioxidant, pro-energetic properties as well as a comprehensive protective and reparative action.

Carnitine plays a key role in intermediate metabolism since it maintains normal mitochondrial function and adequate cellular energy supply, having the ability to transport long-chain fatty acids from cytosol to mitochondrial matrix, where the oxidation of fatty acids occurs (24). Mitochondrial damages or mutations in mitochondrial DNA have been associated with a variety of internal ear disorders, including hearing loss caused by acoustic trauma. L-acetylcarnitine increases mitochondrial energy production and restores cardiolipin and carnitine levels, seriously depleted in tissues exposed to oxidative stress. In addition, L-acetylcarnitine can increase the activity of some mitochondrial respiratory enzymes, improve mitochondrial DNA transcription, restore mitochondrial metabolite transport and promote protection of mitochondrial membrane integrity. Systemic administration of L-acetylcarnitine has been shown to drastically reduce experimentally induced hearing loss (25). Other long-demonstrated functions of carnitine are the preservation of cell membrane integrity, the stabilization of the physiological relationship between coenzyme A and acetyl-CoA in mitochondria and the reduction of lactate production (24). L-acetylcarnitine also exerts systemic anti-inflammatory action, with a significant reduction in C-reactive protein levels after oral supplementation (26). Oral supplementation of L-acetylcarnitine exerts anti-inflammatory effects (27) associated with significant antioxidant action on blood vessels. Antioxidant action can decrease oxidative stress markers and increase antioxidant enzymes (28).

Ginkgo biloba extract can increase cerebral and vestibular blood flow (29,30) by improving hemorheology (31). Ginkgolide B, a component of *Ginkgo biloba* extract, is a platelet-activating factor (PAF) antagonist, a well-known phospholipid mediator derived from numerous inflammatory cells, involved in platelet aggregation, in thrombus formation, in early stage atherogenesis and in capillary permeability alterations (32). *Ginkgo biloba* extract enhances neuronal plasticity (33), mitochondrial function and energy metabolism (34) and seems to regulate glucose consumption, resulting in an increase in cellular ATP levels (35). In addition, *Ginkgo biloba* extract has an antioxidant effect and protects neurones and blood vessels from oxidative damage (36). Finally, ginkgolides inhibit cortisol release in response to stress, leading to a decrease in peripheral benzodiazepine receptor expression in the adrenal cortex (34). Randomised placebo-controlled trials have also demonstrated its efficacy in the treatment of vestibular and non-vestibular vertigo (37). In a randomized, double-blind, placebo-controlled clinical trial, *Ginkgo biloba* extract has been shown to be as effective as betahistine in the treatment of vertigo syndromes of multiple origins (38).

Vitamin B12 is involved in many fundamental cellular events, such as DNA synthesis. Its deficiency negatively affects folic acid metabolism leading to an increase in homocysteine levels. This is an important risk factor for hyperhomocysteinemia, atherosclerosis and cardiovascular diseases. Oxidative balance in tissues is disrupted in patients with hyperhomocysteinemia, augmenting the oxidative damage. Vitamin B12 is also known to be involved in myelination and nerve regeneration. Human trials and basic research have shown that deficiency can cause multiple neurological deficits.

Moreover, studies have shown that neuronal regeneration is significantly greater in patients receiving vitamin B12 when its plasma levels are higher than physiological levels (39).

Data available in the literature therefore suggest that L-acetylcarnitine, *Ginkgo biloba* extract and vitamin B12 provide significant support in the treatment of patients with vestibular diseases. Our study hypothesis is the assumption that the administration of a tested nutraceutical product (Brainil®), containing these three ingredients, in combination with vestibular rehabilitation physical therapy can contribute to vestibular rehabilitative therapy success.

This trial is a pilot study aiming to assess project adequacy and to get all the information necessary to determine the size of the statistical sample needed to verify the significance of the results obtained. The results obtained in this pilot study seem to favourably support the hypothesis formulated. In fact, it emerged that the increase in the RI value was statistically significant in both treatment groups, but in the Brainil group the return to normal values occurred in more patients than in the control group, with a difference between the two groups that was at the limit of statistical significance. The reduction in the CI value was statistically significant only in the Brainil group, showing superiority of Brainil® combination and vestibular rehabilitation compared with vestibular rehabilitation alone. A greater number of patients in the Brainil group returned to normal cVEMP values than in the control group.

The increase in VOR gain was statistically significant in both treatment groups, but outcomes in the Brainil group were statistically superior to those obtained in the control group. The number of patients who returned to normal VOR values in the Brainil group was greater than that in the control group. Saccade cancellation involved more patients in the Brainil group than in the control group. Finally, the results from the DHI-I questionnaire showed statistically significant improvement in both treatment groups but, in the Brainil group, patients' perception of disability at the end of treatment was significantly lower than in the control group.

Then, values obtained by comparing the two patient groups showed a more favorable trend resulting from the use of Brainil®. It is easy to understand that these differences could become even clearer as the number of patients in the two compared groups increases.

Conclusions

Literature datasets allow us to affirm that treatment of vestibular disorders with Brainil® compound ingredients can have beneficial effects. The results of the present study seem to confirm the hypothesis formulated: the combination of Brainil® intake and vestibular rehabilitation seems to result in a synergistic action between the two therapeutic solutions, useful to improve clinical results. In fact, the results of the present pilot study, partially conditioned by the low number of patients treated, highlight that the combination of compound based on L-acetylcarnitine, *Ginkgo biloba* extract and vitamin B12 (Brainil®) and vestibular rehabilitation physical therapy can have a significantly higher effect on quantitative recovery of VOR gain as well as on the normalization of stabilometric parameters (index of cervical interference) compared to physical rehabilitative therapy alone. Moreover this results in higher clinical outcomes with regard to the other parameters compared to physical rehabilitative therapy alone.

Early and correct differential diagnosis is essential in patients with VN, because similar clinical pictures can have different, sometimes very serious, etiologies. Physical rehabilitative therapy can really help patients recover vestibular function. The combination of Brainil® and vestibular therapy can produce additional beneficial outcome compared to vestibular rehabilitation alone.

After 90 consecutive days of treatment with Brainil®, no adverse effect was reported and no patient discontinued therapy, confirming its high tolerability.

A quantitative and qualitative increase in preliminary data obtained to date is advisable in order to improve comprehensive VN management.

References

1. Furuta Y, Takasu T, Fukuda S, Inuyama Y, Sato KC, Nagashima K. Latent herpes simplex virus type 1 in human vestibular ganglia. *Acta Otolaryngol Suppl* 1993;503:85-89.
2. Hirata Y, Gyo K, Yanagihara N. Herpetic vestibular neuritis: an experimental study. *Acta Otolaryngol* 1995;Suppl 519(Suppl):93-96.
3. Gacek R, Gacek M. The three faces of vestibular ganglionitis. *Ann Otol Rhinol Laryngol* 2002;111:103-114.
4. Theil D, Arbusow V, Derfuss T et al. Prevalence of HSV-1 LAT in human trigeminal, geniculate, and vestibular ganglia and its implication for cranial nerve syndromes. *Brain Pathol* 2001;11:408-413.
5. Baloh RW, Ishyama A, Wackym PA, Honrubia V. Vestibular neuritis: clinical–pathologic correlation. *Otolaryngol Head Neck Surg* 1996;114:586-592.
6. Chung JH, Lee SH, Park CW, Jeong JH, Shin JH. Clinical Significance of Arterial Stiffness and Metabolic Syndrome Scores in Vestibular Neuritis. *Otol Neurotol* 2017;38(5):737-741.
7. Sekitani T, Imate Y, Noguchi T, Inokuma T. Vestibular neuronitis: epidemiological survey by questionnaire in Japan. *Acta Otolaryngol Suppl* 1993;503:9-12.
8. Adamec I, Krbot Skoric M, Handzic J, Habek M. Incidence, seasonality and comorbidity in vestibular neuritis. *Neurol Sci* 2015;36:91-95.
9. Theil D, Arbusow V, Derfuss T et al. Prevalence of HSV-1 LAT in human trigeminal, geniculate, and vestibular ganglia and its implication for cranial nerve syndromes. *Brain Pathol* 2001;11:408-413.
10. Hall CD, Herdman SJ, Whitney SL et al. Vestibular rehabilitation for peripheral vestibular hypofunction: an evidence-based clinical practice guideline: from the American Physical Therapy Association Neurology section. *J Neurol Phys Ther* 2016;40:124-155.
11. Heinrichs N, Edler C, Eskens S, Mielczarek MM, Moschner C. Predicting continued dizziness after an acute peripheral vestibular disorder. *Psychosom Med* 2007;69:700-707.
12. Godemann F, Siefert K, Hantschke-Bruggemann M, Neu P, Seidl R, Strohle A. What accounts for vertigo one year after neuritis vestibularis: anxiety or a dysfunctional vestibular organ? *J Psychiatr Res* 2005;39:529-534.
13. Bergenius J, Perols O. Vestibular neuritis: a follow-up study. *Acta Otolaryngol* (Stockh) 1999;119:895-899.
14. Herdman S, Whitney S. Physical therapy treatment of vestibular hypofunction. In: Herdman S, Clendaniel RA, editors. Vestibular rehabilitation. Philadelphia: F.A. Davies; 2014. pp. 394-431.
15. Lacour M, Tighilet B. Plastic events in the vestibular nuclei during vestibular compensation: the brain orchestration of a "deafferentation" code. *Restor Neurol Neurosci* 2010;28:19-35.
16. Deveze A, Bernard-Demanze L, Xavier F et al. Vestibular compensation and vestibular rehabilitation. Current concepts and new trends. *Neurophysiol Clin* 2014;44(1):49-57.
17. Marioni G, Fermo S, Zanon D, Broi N, Staffieri A. Early rehabilitation for unilateral peripheral vestibular disorders: a prospective, randomized investigation using computerized posturography. *Eur Arch Otorhinolaryngol* 2013;270:425-435.
18. Teggi R, Caldirola D, Fabiano B, Recanati P, Bussi M. Rehabilitation after acute vestibular disorders. *J Laryngol Otol* 2009;123:397-402.
19. Venosa AR, Bittar RS. Vestibular rehabilitation exercises in acute vertigo. *Laryngoscope* 2007;117:1482-1487.
20. Kammerlind AS, Ledin TR, Odkvist LM, Skargren EI. Effects of home training and additional physical therapy on recovery after acute unilateral vestibular loss: a randomized study. *Clin Rehabil* 2005;19:54-62.
21. Tokle G, Mørkved S, Bråthen G, Goplen FK, Salvesen Ø, Arnesen H, Holmeslet B, Nordahl SHG, Wilhelmsen KT. Efficacy of Vestibular Rehabilitation Following Acute Vestibular Neuritis: A Randomized Controlled Trial. *Otol Neurotol* 2020; 41(1):78-85.
22. Marcelli V. Vestibologia clinica. Casi clinici e test diagnostici. Edizioni Materia Medica. Pavia 2013.
23. Chabbert C. Principles of vestibular pharmacotherapy. *Handb Clin Neurol*. 2016;137:207-18.
24. Gnoni A, Longo S, Gnoni GV, Giudetti AM. Carnitine in Human Muscle Bioenergetics: Can Carnitine Supplementation Improve Physical Exercise? *Molecules* 2020;25(1). pii: E182.
25. Kopke RD. Pharmacological approaches to the prevention and treatment of cochlear injury due to noise. *Audiological Medicine* 2007;5(1):66-80.
26. Sahebkar A. Effect of L-carnitine Supplementation on Circulating C-reactive Protein Levels: A Systematic Review and Meta-Analysis. *J Med Biochem* 2015;34(2):151-159.
27. Aldemir M, Pektas MB, Parlar AI et al. L-Carnitine Supplementation Reduces Short-Term Neutrophil-Lymphocyte Ratio in Patients Undergoing Coronary Artery Bypass Grafting. *Int Surg* 2015;100(7-8):1160-1168.
28. Lee BJ, Lin JS, Lin YC, Lin PT. Effects of L-carnitine supplementation on oxidative stress and antioxidant enzymes activities in patients with coronary artery disease: a randomized, placebo-controlled trial. *Nutr J* 2014;13:79.
29. Heiss WD, Zeiler K. Medikamentöse Beeinflussung der Hirndurchblutung [The influence on drugs on cerebral blood flow]. *Pharmakotherapie* 1978; 1(3):137-144.
30. Maass B, Silberzahn J, Simon R. Zur Wirkung von Ginkgo-biloba-Extrakt (Tebonin) auf die Wasserstoff-Auswaschvorgänge an der Cochleabasis unter hypotensiver Ischämie. *Extracta Otorhinolaryngologica* 1987;9(5):1 69-172.
31. Huang S, Jeng C, Kao S, Yu JJ, Liu D. Improved haemorrheological properties by Ginkgo biloba extract (Egb 761) in type 2 diabetes mellitus complicated with retinopathy. *Deutsches Arzteblatt International* 2004;23(4):615-621.
32. Smith PF, Maclennan K, Darlington CL. The neuroprotective properties of the Ginkgo biloba leaf: a review of the possible relationship to platelet-activating factor (PAF). *J Ethnopharmacol* 1996;50:131-139.
33. Tchantchou F, Xu Y, Wu Y, Christen Y, Luo Y. EGb 761 enhances adult hippocampal neurogenesis and phosphorylation of CREB in transgenic mouse model of Alzheimer's disease. *TheFASEB Journal* 2007;21(10): 2400-2408.
34. Abdel-Kader R, Hauptmann S, Keil U et al. Stabilization of mitochondrial function by Ginkgo biloba extract (EGb 761). *Pharmacological Research* 2007;56(6):493-502.
35. DeFeudis FV, Drieu K. Ginkgo biloba extract (EGb 761) and CNS functions: basic studies and clinical applications. *Curr Drug Targets* 2000;1(1):25-58.
36. Abdel-Wahab BA, Abd El-Aziz SM. Ginkgo biloba protects against intermittent hypoxia-induced memory deficits and hippocampal DNA damage in rats. *Phytomedicine* 2012;19(5):444-450.
37. Hamann KF. Ginkgo special extract EGb 761 in vertigo: a systematic review of randomised , double-blind, placebocontrolled clinical trials. *The Internet Journal of Otorhinolaryngology* 2007;6(2):258-263.
38. Sokolova L, Hoerr R, Mishchenko T. Treatment of Vertigo: A Randomized, DoubleBlind Trial Comparing Efficacy and Safety of Ginkgo biloba Extract EGb 761 and Betahistine. *Int J Otolaryngol* 2014;2014:682439.
39. Sahin MM, Ugur MB, Karamert R et al. Evaluation of Effect of Garlic Aged Extracts and Vitamin B12 on Noise-Induced Hearing Loss. *Noise Health* 2018;20(97): 232-239.

PHARMALINE
More than Health

Brainil®

30 | COATED DIVISIBLE TABLETS

L-Acetylcarnitine
Ginkgoselect® Plus Phytosome®
Vitamin B12

- **Contributes to the normal functioning of the nervous system**
- **Supports physiological function of the microcirculation**
- **Exerts an antioxidant action**

Ingredients	for 1 tablet	for 2 tablets
L-acetylcarnitine HCl	590 mg	1180 mg
of which L-acetylcarnitine	500 mg	1000 mg
equal to L-carnitine	396,5 mg	793 mg
Ginkgoselect® Plus Phytosome®	80 mg	160 mg
Vitamin B12	16,5 mcg*	33 mcg♦

NRV = Nutrient Reference Value *660% NRV ♦1320% NRV

Recommended dosage:
1-2 tablets per day.

GLUTEN FREE

Pharma Line S.r.l. - Via Agostino Bertani, 2 - 20154 Milan ITALY

www.pharma-line.it