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The Consumption of Alanerv® Nutritional Supplement and the Dynamic of Some Inflammatory Markers in Post-Acute Stroke Patients Undergoing Rehabilitation

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ABSTRACT

Objectives: *Stroke is followed by an inflammatory response lasting up to several months. Moreover, many of the stroke-related comorbidities (i.e., diabetes mellitus, dyslipidemia, cardiovascular disease, and atherosclerosis) are characterized by an pro-inflammatory status.*

Material and Methods: *We designed this pilot study to evaluate the relation between the consumption of a nutritional supplement (ALAnerv®) and the dynamic of the inflammatory status in post-acute stroke patients undergoing rehabilitation. The study population comprised 28 patients which were assigned into two study groups, named (-) ALA and (+) ALA. All subjects followed the same rehabilitation program. There were no significant differences in respect to the standard medication between the groups. Moreover, patients from the (+) ALA group received ALAnerv® for two weeks (2 pills/day). We assessed IL-1α, IL-6, TNF-α, sICAM-1, and myeloperoxidase in blood samples taken at the beginning and at the end of the study period.*

Outcomes: *In the (+) ALA group only IL-1α (- 9.9% ± 3.7, P = 0.013) and IL-6 (- 26.5% ± 8.2, P = 0.003) significantly decreased during the study period. The multiple regression analysis indicated that the ALAnerv® treatment was responsible for the significant decrease of IL-6 level (P = 0.008). Moreover, the percentage of IL-6 variation between the study groups reached statistical significance (8.4% ± 11.5 vs. – 26.5% ± 8.2, P = 0.034).*

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Conclusions: *These results indicate that ALAnerv® could be beneficial for the correction of the inflammatory status in post-acute stroke patients and underline the need of a longer treatment period with a higher dose.*

Keywords: stroke, IL-1α, IL-6, lipoic acid, rehabilitation, ALAnerv®

INTRODUCTION

Stroke is the second cause of mortality worldwide, putting an important financial burden due to the high costs needed for patients' recovery and rehabilitation (1). Moreover, it is estimated that until 2030, this pathological condition will became the fourth cause of disability worldwide (2). There are two types of stroke, ischaemic and haemorrhagic, the former having an incidence of 80-85% (3).

The inflammatory status of a subject is considered to be a risk factor for both the stroke itself, as well as for the stroke recurrence. Most of the pathological conditions that represent stroke risk factors are associated themselves with a pro-inflammatory state (4).

As a consequence of the cerebral ischemia, different types of cells from the nervous tissue start to release several molecular agents (cytokines [IL-1β, IL-4, IL-6, IL-10, TNF-α], chemokines [MCP-1], interferons, MMPs) (5,6). Endothelial cells of the cerebral vessels are stimulated by some of the aforementioned molecules to express adhesion molecules (selectins, immunoglobulins, and integrins) which will enable the recruitment and accumulation of the activated leukocytes from the circulation (6). This is possible because MMPs, alongside with other proteases, contribute to the degradation of the tight junctions and the disintegration of the BBB (7).

High values for C-reactive protein, white blood cell count and erythrocytes' sedimentation rate were found in humans even after 90 days from stroke (8). This pro-inflammatory status is most likely caused by a combination of the immune activation generated by the cerebrovascular event and the pre-existing inflammatory condition. ❑

OBJECTIVES

The present pilot study was designed in order to evaluate the potential of the ALAnerv® nutritional supplement to help the correction of the inflammatory status in post-acute stroke patients. To achieve this purpose we select some inflammatory markers (IL-1α, IL-6, TNF-α, sICAM-1, and MPO) and monitored their dynamics for a period of two weeks in two groups of patients, one of which received 2 pills/day of ALAnerv®, the other being the control group. ❑

MATERIAL AND METHODS

Design and subjects

The study population comprised 28 post-acute stroke patients. They were randomly divided into (-) ALA and (+) ALA groups, each of which consisted of 14 subjects (7 females/7 males). We used as inclusion criterion for both groups the diagnostic of an ischemic or hemorrhagic stroke in the previous 90 days before the enrolment. Cancer, chronic renal failure, chronic inflammatory, autoimmune and haematological disorders, smoking and chronic alcohol consumption were considered as exclusion criteria. Also, patients who were under treatment with vitamins and anti-inflammatory drugs during the two months preceding the beginning of the study and those with a previous cerebrovascular event (cerebral haemorrhage, hemorrhagic infarct, transient ischemic attack) were excluded from the study. We recorded the medication used by all the subjects.

All the patients were hospitalized during the study period and underwent a standard rehabilitation program, receiving medication accordingly with their pre-existing comorbid states. Moreover, patients from the (+) ALA group received 2 pills/day of ALAnerv® during this period. The efficiency of the rehabilitation program was evaluated using the Barthel Index (BI) scale (9).

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving patients were approved by the ethics review boards of the National Institute of Rehabilitation, Physical Medicine and Balneoclimatology and „Elias" Emergency Hospital, Bucharest (Romania). Written informed consent was obtained from all patients or from their relatives.

ALAnerv® composition description

According to the manufacturer specification sheet, one soft gelatine capsule of ALAnerv® contains: α-lipoic acid (300 mg), Borago officinalis (300 mg) which contains 180 mg polyunsaturated fatty acids (linoleic acid and gamma-linolenic acid), D-α-tocopherol on sun flower oil basis (11.177 mg) which contains 7.5 mg vitamin E, thiamine mononitrate 1.259 mg (equivalent of 1.05 mg vitamin B1), riboflavin 1.320 mg (equivalent of 1.2 mg vitamin B2), calcium pantothenate 5.396 mg (equivalent of 4.5 mg vitamin B5), pyridoxine hydrochloride 2.010 mg (equivalent of 1.5 mg vitamin B6), selenomethionine 0.069 mg with 25 μg selenium, fatty acids triglycerides (60 mg), magnesium stearat (14 mg), polyglycerol oleate (10 mg), soya oil and soya lecithine complex (6 mg), food gelatin (177.940 mg), glycerol (82 mg), titanium dioxide (1.520 mg), iron red oxide (0.130 mg).

Blood samples

Whole blood was colected after an overnight fasting of 8-10 hours. Serum and plasma were isolated immediately and stored in 1.5 mL Eppendorf tubes at -80°C until analysis.

Inflammatory markers

IL-1α, IL-6, TNF-α, and sICAM-1 were assessed using special kits (DRG Diagnostics, Germany). The detection limits and the intra assays CV% for these kits are as follows, as specified by the producers: IL-1α (1.1 pg/mL; 5.4%), IL-6 (2 pg/mL; 4.2%), TNF-α (0.7 pg/mL; 6.6%), and sICAM-1 (2 ng/mL; 7.8%). MPO was assessed using also a kit with the detection limit and the intra assays CV% of 3.3 μg/L and 8.5%, respectively, as specified by the producer (Mercodia, Uppsala, Sweden). An ELISA reader (Sunrise Absorbance Reader, Tecan) was used for these assays.

Statistics

All the data are given as mean values and standard error of the mean (SEM). For each biochemical parameter, Wilcoxon and Mann-Whitney tests were used to compare the means between the two moments of the study and the percentage of variation, respectively. Chi-square test was used to evaluate the differences between the two groups in respect to comorbidities incidence and medication. Multiple regression analysis was performed to evaluate the relations between independent variables (baseline values of the assessed parameters, ALAnerv® treatment, and incidence of diabetes mellitus) and a dependent variable (discharge values of the assessed parameters). In order to avoid inter-assay variability, the statistical analysis was performed using the values obtained after dividing individual values with the mean value of each group. Statistical analysis was performed using the GraphPad InStat 3 software. A value of $P < 0.05$ was considered statistically significant. ❑

OUTCOMES

We enrolled 28 post-acute stroke patients which were hospitalized for a period of two weeks for rehabilitation procedures. We assigned these subjects into the previously mentioned groups, (-) ALA and (+) ALA. The demographic, comorbidities and medication used by the participants in the study are presented in Table 1. There are no statistical differences between the two groups, except for the incidence of diabetes mellitus.

Table 2 presents the values of the assessed inflammatory markers. As previously stated in the Statistical analysis section, for each parameter we used the values obtained after dividing each individual data with the mean of the corresponding group. In this way we intended to minimize the inter-assay variability, as for each biochemical marker not all samples were assessed using the plate from the same kit. In (+) ALA group only the concentrations of IL-1α (- 9.9% ± 3.7, $P = 0.013$) and IL-6 (- 26.5% ± 8.2, $P = 0.003$) reached statistical significance, while in (-) ALA group none of the parameters reached the statistical significance. Moreover, the percentage of variation between the two study groups reached statistical significance only for the IL-6 (8.4% ± 11.5 vs. – 26.5% ± 8.2, $P = 0.034$). The multiple regression analysis indicated that the ALAnerv® treatment was responsible only for the significant decrease of IL-6 level ($B = - 0.26$, $SEM = 0.09$, $P = 0.008$) during the study.

As for the rehabilitation efficiency, we found that the BI values significantly increased in both groups ((-) ALA, 7.2% ± 1.2, $P < 0.001$; (+) ALA, 48.4% ± 17.7, $P < 0.001$), the improvement being more pronounced in the (+) ALA group (7.2% ± 1.2 vs. 48.4% ± 17.7, P =

	(-) ALA	(+) ALA	P value
Demographic characteristics			
Age (years)	67.1 ± 2.9	64.0 ± 2.9	NS[a]
Sex ratio (females/males)	7/7	7/7	NS[b]
Time from stroke (range, days)	36.1 ± 5.5 (12-86)	48.6 ± 7.4 (21-91)	NS[a]
Vascular risk factors			
Hypertension, N (%)	11 (78.6)	10 (90.9)	NS[b]
Diabetes mellitus, N (%)	2 (14.3)	6 (54.6)	0.044[b]
Dyslipidemia, N (%)	7 (50)	5 (45.5)	NS[b]
Coronary ischemic disease, N (%)	4 (28.6)	5 (45.5)	NS[b]
Carotid atherosclerosis, N (%)	7 (50)	3 (27.3)	NS[b]
Medication			
Statins, N (%)	11 (78.6)	8 (72.7)	NS[b]
Antiplatelet agents, N (%)	3 (21.4)	3 (27.3)	NS[b]
Antithrombotic agents, N (%)	6 (42.9)	4 (36.4)	NS[b]
Anticoagulant agents, N (%)	2 (14.3)	4 (36.4)	NS[b]
Antidepressive agents, N (%)	6 (42.9)	9 (81.8)	NS[b]
Antiacids, N (%)	7 (50)	8 (72.7)	NS[b]
Antihypertensive agents, N (%)	7 (50)	8 (72.7)	NS[b]
ACE inhibitors, N (%)	7 (50)	6 (54.6)	NS[b]
Beta blockers, N (%)	9 (64.3)	7 (63.6)	NS[b]
Acetylsalicilic acid, N (%)	7 (50)	3 (27.3)	NS[b]

TABLE 1. Demographic, comorbidities and medication of the study groups.

a Mann-Whitney test; b Chi-square test; NS, non significant.

		Baseline	Discharge	Change (%)	P value[a]	P value[b]
IL-1α (pg/mL)	(-) ALA	1.03 (0.06)	0.99 (0.06)	- 1.9 (6.7)	NS	NS
	(+) ALA	1.05 (0.15)	0.94 (0.13)	- 9.9 (3.7)	0.013	
IL-6 (pg/mL)	(-) ALA	1.01 (0.11)	0.99 (0.07)	8.4 (11.5)	NS	0.034
	(+) ALA	1.28 (0.27)	0.72 (0.03)	- 26.5 (8.2)	0.003	
TNF-α (pg/mL)	(-) ALA	1.02 (0.08)	0.98 (0.10)	- 2.1 (7.5)	NS	NS
	(+) ALA	1.12 (0.13)	0.88 (0.07)	- 6.5 (13.6)	NS	
sICAM-1 (ng/mL)	(-) ALA	1.01 (0.07)	0.99 (0.05)	0.7 (4.1)	NS	NS
	(+) ALA	1.02 (0.05)	0.98 (0.05)	- 2.6 (4.5)	NS	
MPO (μg/L)	(-) ALA	1.01 (0.10)	0.99 (0.11)	0.8 (8.5)	NS	NS
	(+) ALA	1.07 (0.17)	0.93 (0.17)	- 7.8 (11.8)	NS	

TABLE 2. The dynamic change in mean concentration of inflammatory markers according to the absence or the presence, respectively of ALAnerv®.

a baseline vs. 2 weeks (Wilcoxon paired test), b difference in change (%) between baseline vs. 2 weeks (Mann-Whitney test), NS, non significant.

0.019). According to the regression analysis, both ALAnerv® treatment and the baseline BI values significantly affected the BI values at the discharge moment. ❑

DISCUSSIONS

The post-stroke inflammation can last up to several months leading to brain injuries through different pathways (5,8). Moreover, there are some pro- and anti-inflammatory cytokines that could indicate the potential prognosis after a particular sub-type of stroke. For example, one study indicated that IL-6 concentration in blood samples from ischemic stroke patients was associated with a larger afflicted cerebral tissue and a poorer outcome after one year (10). On the other hand, another study indicated that the level of IL-6 at admission is associated with early clinical deterioration (11). Moreover, a clinical trial indicated that IL-6, and TNF-α were associated with the risk of recurrent stroke (12). Also, endothelial activation revealed by the increased values of sICAM-1 in ischaemic stroke patients at admission is related to a poor prognosis (13).

With all of this in mind we designed the present pilot study in order to investigate the ability of the nutritional supplement ALAnerv® to improve the inflammatory status in post-acute stroke patients. Despite the fact that ALAnerv® is a complex mixture of compounds, at least three of these could help to the correction of the inflammatory status: LnA, GLA and LA.

LnA is an essential fatty acid and it is the substrate for the Δ6 desaturase expressed in a

variety of human cells (14). The product of this desaturase is GLA which undergoes further elongation to DGLA, the latter being incorporated into the plasma membrane phospholipids (14). DGLA released through the action of phospholipase A2 became substrate for three enzymes: COX-1/-2, 15-LOX and Δ5 desaturase (15). The latter converts DGLA to AA, the direct precursor of the 2-series prostaglandins and 4-series leukotriens known for their pro-inflammatory properties. In humans, the Δ5 desaturase has a very limited activity, and as a consequence just a very small amount of DGLA is transformed to AA (16). The neutrophils from subjects of which diet was supplemented for three weeks with 3 g GLA/day synthesized smaller amounts of LTB4 and PAF.

DGLA can also be transformed to prostaglandins from 1-series (PGE1, TxA1) by COX-1/-2 or can be oxygenated by 15-LOX to the 15-(S)-hydroxy-8,11,13-eicosatrienoic acid (15-HETrE) (17). Moreover, 15-HETrE is a potent inhibitor of the 5-LOX, blocking thus the synthesis of LTB4 (18).

Another component of ALAnerv® with proven anti-inflammatory properties both *in vivo* and *in vitro* is α-tocopherol (19). Its ability to downregulate pro-inflammatory cytokines (IL-1, IL-6, TNF-α), the chemokine IL-8, as well as hsCRP levels is well documented (20).

Not the least, LA is also a well known anti-inflammatory compound. For example, the accumulation of the pro-inflammatory prostaglandins PGE2 and PGF2α in rats' brains treated with a mixture of pesticides was decreased in a dose-dependent manner by LA (21). In another study, using also rats as animal model, an acute and a chronic inflammatory response was induced with carrageenan and cotton pellet, respectively (22). In both situations the use of LA was associated with a decreased activity of MPO, as a marker of activated neutrophils.

As previously mentioned, IL-1α and IL-6 are the only cytokines for which statistical significance was reached in (+) ALA group. On the other hand, the multiple regression analysis model indicated that the treatment with ALAnerv® significantly influenced only IL-6. Moreover, IL-6 is the only parameter for which the percentage of variation between the two groups attained statistical significance (P = 0.034). For all the other inflammatory markers that we assessed we found decreasing trends in both groups, but with a greater percentage of change in the (+) ALA group. This led us to the conclusion that a major drawback of our pilot study was the short time period used which made impossible a biochemical response of all the assessed markers.

In conclusion, the present study opens the possibility of using ALAnerv® for a longer time period to help the correction of the inflammatory status in post-acute stroke patients. Moreover, this nutritional supplement could be beneficial for long time consumption as it is a source of some vitamins, selenium and an essential fatty acid.

Conflict of interest: none declared.

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Abbreviations list

AA = arachidonic acid (cis-5,cis-8,cis-11,cis-14-eicosatetraenoic acid)
BBB = blood-brain barrier
COX = cyclooxygenase
DGLA = dihomo-gamma-linolenic acid (cis-8,cis-11,cis-14-eicosatrienoic acid)
GLA = γ-linolenic acid (cis-6,cis-9,cis-12-octadecatrienoic acid)
LA = lipoic acid ((R)-5-(1,2-dithiolan-3-yl)pentanoic acid)
LnA = linoleic acid (cis-9,cis-12-octadecadienoic acid)
LOX = lipoxygenase
LTB4 = leukotriene B4
MCP-1 = monocyte chemoattractant protein-1
MMPs = matrix metalloproteinases
MPO = myeloperoxidase
PAF = platelet-activating factor
sICAM-1 = soluble intercellular adhesion molecule 1.

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