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The Nutraceutical Potential of Omega-3 Alpha-Linolenic Acid in Reducing the Consequences of Stroke

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Running title: ALA importance in combating stroke

Highlights

ALA was evaluated as a nutraceutical in rodent model of ischemic stroke.

ALA supplementation by modification of the daily diet prevents mortality and cerebral damage.

ALA stimulates neuronal protection, neuroplasticity, and brain artery vasodilation.

ALA stimulates brain preconditioning mechanisms.

We propose the novel concept of brain preconditioning by nutraceuticals against stroke.

Abbreviations

1 ALA: Alpha-Linolenic Acid

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3 ANSES: Agence Nationale pour la Sécurité et la Santé

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5 BDNF: Brain Derived Neurotrophic Factor

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7 CVD: CardioVascular diseases

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9 DHA: DocosaHexaenoic Acid

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11 EPA: EicosaPentaenoic Acid

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13 LA: linolenic acid

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15 LC omega-3: long-chain omega-3 (eg, mainly EPA and DHA)

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17 MCAO: middle cerebral artery occlusion

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19 PUFA: polyunsaturated fatty acids

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21 SNAP-25: Synaptosomal-Associated Protein 25

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23 VAMP-2: Vesicle-Associated Membrane Protein 2

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25 VGLUT1: Vesicular Glutamate Transporter 1

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27 VGLUT2: Vesicular Glutamate Transporter 2

Abstract:

Stroke is a worldwide major cause of mortality and morbidity. Preclinical studies have identified over 1000 molecules with brain-protective properties. More than 200 clinical trials have evaluated neuroprotective candidates for ischemic stroke yet, to date almost all failed, leading to a re-analysis of treatment strategies against stroke. An emerging view is to seek combinatory therapy, or discovering molecules able to stimulate multiple protective and regenerative mechanisms. A pertinent experimental approach to identify such candidates is the study of brain preconditioning, which refers to how the brain protects itself against ischemia and others stress-inducing stimuli. The recent discovery that nutrients like alpha-linolenic acid (ALA is an essential omega-3 polyunsaturated fatty acid required as part of our daily diet), may be an efficient brain preconditionner against stroke fosters the novel concept of brain preconditioning by nutraceuticals.

This review stresses the underestimated role of nutrition in preventing and combating stroke. Although there is a consensus that increased consumption of salt, fatty foods and alcoholic beverages may promote pathologies like hypertension, obesity and alcoholism - all of which are well known risk factors of stroke - few risk factors are attributed to a deficiency in an essential nutrient in the diet. The ALA deficiency observed in the Western modern diets may itself constitute a risk factor.

This review outlines how ALA supplementation by modification of the daily diet prevented mortality and cerebral damage in a rodent model of ischemic stroke. It also describes the pleiotropic ability of ALA to trigger responses that are multicellular, mechanistically diverse, resulting in neuronal protection, stimulation of neuroplasticity, and brain artery vasodilation. Overall, this review proposes a promising therapeutic opportunity by integrating a nutritional-based approach focusing on enriching the daily diet in ALA to prevent the devastating damage caused by stroke.

Keywords:

Ischemic stroke - brain preconditioning - neuroprotection - omega-3 polyunsaturated fatty acids – functional food - synaptogenesis - neurogenesis - nutraceutical - enriched diet

1. Stroke is a worldwide main cause of mortality and morbidity, lacking therapeutic options

Stroke is a devastating disease in developed and 3rd world countries, due to its high incidence, its brutal impact on the patient and its relatives, and the lack of therapeutic options. On average, someone has a stroke every 40 and 90 seconds in the United States and Europe, respectively [1, 2]. Annually, 15 million people worldwide suffer a stroke. The total number of stroke deaths is estimated at a half million people per year in European Union and is three time higher in the U.S. Of these, 30% die and another 30% are left permanently disabled, placing a tremendous burden on family and community. The estimated cost of stroke for 2010 was \$74 billion and €64 billion in the U.S and Europe, respectively [2, 3].

For the public, stroke is better known as brain attack, because it strikes in 85% of the cases by disrupting the blood flow to part of the brain due to occlusion of a blood vessel feeding the brain. Stroke is therefore a hypoxic-ischemic injury, whose pathophysiology involves glutamate, the major physiological excitatory neurotransmitter in the brain. The lack of oxygen and glucose causes a massive release of glutamate from neurons, and the overactivation (excitotoxicity) of ionotropic glutamate receptors, predominantly the N-methyl-D-aspartate (NMDA) glutamate receptor subtype. This results in accumulation of intracellular calcium, which in turn triggers deleterious cascades including activation of lytic enzymes, mitochondrial dysfunction, oxidative stress and inflammation [4] in two regions that coexist within the infarct: the necrotic core and the ischemic penumbra, an area surrounding the core where neurons remain on the brink of survival or death for hours [5].

The progresses in understanding its complex interplay of multiple cellular and signaling pathways that alter the neurovascular unit integrity within differentially affected territories allowed identification in preclinical studies of neuroprotective targets or/and drugs blocking the neurotoxic ischemic cascade. Nevertheless, of those tested in clinical trials, all have failed, leaving patients and clinicians without any repertoire of therapeutic opportunities exerting direct protection of the neurons [6]. Consequently, the only approved therapeutic exerts its benefits through the restoration of the blood flow to the brain by blood clot disruption. It is performed by recombinant tissue plasminogen activator (tPA) treatment administered to approximately 5% of stroke patients. On the positive side, three definitive points are worth noting: 1) Efforts have been made over the past decades in high-income countries to control

1 major risk factors like hypertension, diabetes, and high cholesterol, strides which have
2 contributed to stroke mortality reduction. This success is associated with a global
3 improvement in population health. It should be monitored cautiously as it probably represents
4 the tip of the iceberg because stroke mortality represents, at the maximum, a third of the
5 annual first-ever strokes; 2) The failure in translation from experimental models to clinical
6 trials has lead to revisiting the research of strategies against stroke, resulting in a set of drug
7 development criteria, collectively known as the Stroke Therapy Academic Industry
8 Roundtable (STAIR) recommendations [7]; and 3) There is an acceleration in the study of
9 unconventional therapies, with a major theme being to interrogate how the brain protects itself
10 [4, 8].
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20 To summarize, considering the multifactorial nature of stroke, in order to be considered as a
21 good candidate for a clinical trial, a treatment should exhibit multimodal actions on the
22 multiple cell types composing the neurovascular unit. Consequently, an emerging view is that
23 we should preferentially seek and test for drug combinations or multi-therapy, or discovering
24 molecules able to stimulate multiple protective and regenerative mechanisms to fight stroke
25 [9, 10]. A pertinent experimental approach to identify such candidates came from our
26 experience on brain preconditioning through the study of how the brain protects itself against
27 ischemia and others stress stimuli.
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36 **2. The study of the brain preconditioning opens new rational against stroke**

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40 The idea of developing a treatment against stroke inspired by this endogenous protective
41 process is appealing. Preconditioning depends on the stimulation of protection and
42 regeneration against stroke through direct and/or indirect mechanisms, involving multiple cell
43 types, rather than through inhibition of single deleterious events, or targets of most of the
44 conventional neuroprotective approaches. Indeed, brain preconditioning refers to a sublethal
45 toxic stimulus eliciting an endogenous response, which renders the brain remarkably tolerant
46 to a subsequent, normally lethal stimulus of the same insult. Since its original description in
47 the brain [11], the discovery that non-ischemic preconditioners (Figure 1) including various
48 sublethal insults like epilepsy, endotoxins, anoxia, hyperthermia and spreading depression
49 also promote tolerance to ischemia - a phenomenon known as “cross-tolerance” [8, 12, 13] -
50 definitely established that the protective response to brain preconditioners is pleiotropic in
51 nature. A major conceptual roadblock for clinical translation - the requirement of bringing
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1 neurons to the ‘brink of death’ during the sublethal preconditioning challenge [14] - can be
2 circumvented based on the demonstration that brain preconditioning may be
3 pharmacologically/chemically induced by drugs like adenosine or K_{ATP} channel agonists [15].
4 Finally, the recent discovery that nutrients like polyunsaturated fatty acids and
5 lysophospholipids that form part of our daily diet may be efficient brain preconditioners
6 against stroke [16, 17] gave birth to the novel concept of brain preconditioning based on
7 nutraceuticals against stroke [18].
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13 **3. The novel concept of brain preconditioning by nutraceuticals against stroke stresses** 14 **that nutritional importance may go beyond stroke prevention** 15 16

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20 The rationale of supplementation with a non-ischemic preconditioner that could be a natural
21 product - a nutrient defined as a nutraceutical - is based on the experimental demonstration
22 that certain nutraceuticals can act as “natural preconditioners” to increase brain resistance
23 against stroke [18]. Such an approach circumvents issues such as the administration routes
24 and timing issues which thus far have plagued the preconditioning and neuroprotectant fields;
25 such an approach may be more amenable for translation to the clinical arena, since nutrition
26 plays a key role for health and stroke risk in particular. Indeed, most modifiable risk factors of
27 stroke - including hypertension, diabetes, hypercholesterolemia, tobacco use, increased
28 inflammatory markers, dyslipidemia and obesity - often coexist with improper life-style and
29 nutrition, causing imbalances in essential vitamins and nutrients. Striking examples are
30 over-consumption of salt, fatty acids that promotes hypertension and diabetes/obesity that
31 drastically increase the possibility of having a stroke. In contrast, epidemiologic studies
32 usually do not clearly identify a risk factor arising from a deficiency in an essential nutrient in
33 the diet. Nevertheless, an important exception is that many clinical and epidemiologic studies
34 have shown that insufficient dietary intake of fruits and vegetables or of foods containing
35 omega-3 polyunsaturated fatty acids (PUFAs), in the form of Alpha-Linolenic Acid (ALA)
36 and the Long Chain derivatives (LC-n-3), Eicosa-Pentaenoic-Acid (EPA) and Docosa-
37 Hexaenoic-Acid (DHA), represent a risk factor for cardiovascular and cerebral diseases,
38 including coronary heart disease and stroke. In addition, several studies investigating the
39 importance of dietary omega-3 PUFAs, achieved by consumption of seafood (rich in LC-n-3:
40 EPA and DHA) and/or vegetable oils rich in precursor (ALA), proposed omega-3 PUFAs as
41 key support for neurons and brain resistance (for review, see [18, 19]. Therefore, nutritional
42 products with health benefits or supplemented with such protective nutrients seem promising
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2 for reducing the incidence of stroke, encouraging the discovery or characterization of an
3 efficient nutraceutical targeted against stroke.
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5 Unfortunately, no such entity like the STAIR recommendations or any other guidelines exist
6 to directly address nutraceutical discovery, especially in the field of stroke. Nutraceutical is a
7 term coined from nutrition and pharmaceutical without any regulatory definition, and refers
8 to foods or one of its constituents that provides medical or health benefits, including the
9 prevention and/or treatment of a disease [20]. Such an open definition may explain, in part,
10 why the term is often abused for marketing purposes, and several limitations exist in proving
11 the efficiency of a potential candidate to be termed nutraceutical.
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20 The main distinction between nutraceutical and dietary supplements aimed at supporting the
21 body with the required amount of a certain nutrient needed for its proper functioning, is that a
22 nutraceutical should have a proven efficiency against disease [20]. Thus, a supplement may
23 be a nutraceutical; such overlap increases the risk of confusion, especially if the essential
24 distinction of the role of the nutraceutical in combating disease is not well framed. To avoid
25 repeating past mistakes in the field, the best chance for success may be to move forward with
26 a nutraceutical being able to follow, at least for part, the STAIR recommendations. This goal
27 would be achievable by restricting the definition of the term nutraceutical to compounds
28 isolated or purified from foods, and demonstrating that efficiency could be evaluated and used
29 in medicinal forms. In this framework, the following part of the review argues that Alpha-
30 Linolenic Acid conforms to this optimal definition of nutraceutical, by identifying its
31 relevance in protecting from stroke consequence.
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44 **4. Omega-3 supplementation to lower stroke risk: origin of the concept**

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47 A body of evidence shows that omega-3 intake in the adult population of developed countries
48 is far below the recommended Dietary Reference Intake. This deficiency was associated with
49 an increased risk of stroke occurrence and conversely, diets enriched in omega-3, especially
50 through the consumption of fatty fish twice a week, lower the risk of having a stroke [21, 22].
51 The first clinical trials examining the effect of fish oil EPA-DHA supplementation on
52 cardiovascular death were promising, indicating that omega-3 supplementation may be used
53 for lowering stroke risk [23, 24]. That conclusion is disputed by results from recent
54 randomized clinical trials examining the effects of fish oil supplementation on cardiovascular
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disease morbidity and mortality in secondary prevention settings [25, 26]. Nonetheless, continued interest in LC-omega-3 was acknowledged by the American Heart Association and French recommendations, pointing out the necessity to enrich our daily diet in omega-3 fatty acids [27-30].

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Additionally, the few preclinical studies, which have examined the effects of fish oil supplementation in rodent models of ischemic brain injury, suggested a protective action against neuronal damage (for review, see [18, 19, 31]). It is important to note that beneficial effects of LC-omega-3 enriched diets were mainly observed when comparing with diets lacking EPA/DHA. This may explain in part the inconsistency of recent results of clinical trials investigating the effect of omega-3 supplementation: the protection obtained with supplementation with LC-omega-3 may only appear in the case of a severe deficiency in the patient diet. This reinforces the necessity for a clear distinction between nutraceuticals and supplements that should exert a beneficial role compensating a deficiency of the intake. In the context of investigating omega-3 as a nutraceutical, protection of the patients by the enriched diet should also be obtained when comparing to patients fed a diet already containing omega-3 fatty acids of the same nature. Such nutraceutical effect of ALA was discretely suggested in the “Lyon Diet Heart Study”, which reported a reduced rate of recurrence of myocardial infarction, other cardiac events and overall mortality of patients fed an ALA-rich diet as compared to those fed the usual post-infarct diet, known as the “prudent diet” that also contains ALA and other omega-3 fatty acids [32].

5. Alpha-linolenic acid: from supplements to nutraceuticals for stroke?

5.1. Long underestimated, ALA interest as supplement is now re-evaluated

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Indeed, while numerous studies have investigated the beneficial effect of a DHA/EPA-enriched diet, as well as providing characterization of biophysical and functional properties of the nutrient itself, interest in ALA is extremely recent. Several reasons for this early disinterest can be surmised. The first reason was conceptual: DHA is a major constituent of the brain plasma membranes but ALA is not even incorporated at the brain membrane level. In addition, its consumption may not fulfill DHA requirements [33], because of its extremely low efficiency of conversion to DHA [34, 35]. This dogma is now disputed because, as reviewed by Barcelo-Coblijn, when consumed in adequate amounts over time, ALA exerts

1
2 identical effects as DHA in several physiological processes [33]. The second reason resulted
3 from the simplification in approaches, which considered stroke like any other cardiovascular
4 diseases (CVD incidence is lower for an EPA and DHA combination compared to ALA [36,
5 37]). Consequently to the recent controversy on the effect of supplementation performed with
6 EPA or DHA on stroke and CVD and to the growing evidence of the beneficial effect of
7 dietary ALA to protect against CVD, dietary supplementation with ALA regained interest.
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12 A recent body of evidence demonstrates that ALA intake is associated with a reduced risk of
13 stroke in humans. It is worth noting that this effect on stroke was not correlated with a
14 positive effect on coronary heart disease [38]. A reduced risk of stroke was associated with
15 high serum levels of ALA [39]. A lower prevalence of a carotid plaque and inflammation, key
16 steps of atherosclerosis considered as one of the major risk factors of stroke, are associated
17 with elevated intake of ALA [40-42]. The interest in ALA as a supplement is also reinforced
18 by the fact that human do not possess the enzymes for *de novo* synthesis of ALA, in contrast
19 to EPA/DHA; and by the absolute and relative recent changes of omega-6/omega-3 ratio that
20 seems mainly mediated by an increase in LA and a decrease in ALA content in the western
21 diet [43]
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31 32 **5.2. ALA as a nutraceutical for stroke: proof of concept**

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36 Just as for the beneficial effects of an LC-omega-3 enriched diet (observed when comparing
37 with diets deficient in EPA/DHA), a diet rich in ALA from perilla oil increases lifetimes of
38 hypertensive stroke-prone rats compared to a diet rich in LA from sunflower oil [44]. To
39 avoid any confusion between the concept of a supplement and a nutraceutical, we investigated
40 whether an ALA enriched diet from rapeseed oil, a rich source of ALA and the only source of
41 lipids, could reduce brain damage in a mouse model of ischemic stroke (Figure 2), performed
42 by transient occlusion of the middle cerebral artery (MCAO), compared to regular chows
43 supplemented in ALA, EPA and DHA in proportions matching the “murine” recommended
44 intake [45, 46]. In addition, the ALA enriched diet did not contain any LC-omega-3 EPA or
45 DHA. In this paradigm of a 6-week diet enriched in ALA by a factor of three compared to
46 regular chows, a reduced mortality rate and smaller infarct size were observed 24 h after
47 60min of MCAO [47]. The reduction of the infarct volume was similar or better than those
48 observed with high dietary levels of LNC omega-3 provided over the same period of feeding
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1 [48]. Since ALA bioconversion to DHA was negligible over the period of investigation, this
2 neuroprotection may be attributed to a potential nutraceutical effect of ALA.
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5 **5.3. ALA evaluated and used in medicinal forms in rodent model of ischemic stroke**

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9 As reviewed above, the main distinction between nutraceuticals and dietary supplements is
10 that the proven efficiency on disease was observed - at least - in a mice model of stroke. Thus,
11 the additional step was to investigate the hypothesis that the use of ALA could conform to this
12 restricted definition of a nutraceutical, where efficiency could be evaluated when used in
13 medicinal treatment. Evidence has accumulated that bolus injections of ALA and, to a lesser
14 extent, EPA/DHA, is neuroprotective against glutamate-mediated excitotoxicity, a major
15 cause of the initial substantial neuronal damage in animal models of neurodegenerative
16 conditions and neurological injury, like epileptic seizures [49, 50], acute spinal cord injury
17 [31, 51, 52] and focal and global ischemia [17, 50, 52-57].
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27 With regard to acute neuroprotection, pretreatment and “rapid preconditioning” when
28 targeting glutamate excitotoxicity share the same temporal constraint that undermines clinical
29 translation. Nevertheless, testing ALA in such a paradigm provided the first indication of its
30 nutraceutical capacity as a neuroprotectant. The injection of ALA (i.c.v., 10 μ M/5 μ l or i.v.,
31 500 nmol/kg) preserved 80% of the CA1 hippocampal pyramidal neurons in an *in vivo*
32 transient model of global ischemia, a model for which neuronal death is mainly driven by
33 glutamate excitotoxicity, as compared to the 15% survival observed 7 days after 20 min of the
34 4-vessel occlusion [50]. In the MCAO model (Figure 2), intravenous injection of ALA
35 reduced the infarct volume when injected before, but also up to 6 hours after stroke onset,
36 corresponding with post-treatment or postconditioning paradigms [56, 58]. In such a context
37 of standardized evaluation of a neuroprotectant candidate, ALA performed like riluzole, a
38 drug currently in clinical use for amyotrophic lateral sclerosis in a time-frame of intervention
39 compatible within a clinical setting.
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52 When considering nutraceuticals, an interesting aspect offered by the preconditioning and
53 postconditioning approaches is that repetitive activation of these endogenous mechanisms
54 may lead to sustained protection against ischemic stroke [59, 60]. Such an approach with
55 repeated intravenous injections achieved a 3-fold improvement of the long-term survival post-
56 ischemia, while no improvement was observed with a single ALA injection - described to
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reduce post-stroke infarct [56, 58]. The surprising number of neuronal death models and windows of intervention in which beneficial effects of ALA have been described imply multiple temporal and mechanistic benefits.

5.4. The multiple temporal and mechanistic protective effects of ALA

The original mechanistic finding was that ALA can act directly on the neurons by protecting them *in vitro* of neuronal death triggered by excitotoxicity driven by exposure to a Mg²⁺-depleted glycine-supplemented medium (-Mg/+gly) or the addition of an excitotoxic concentration of glutamate [50, 58]. The second was that ALA also acts on another cell type comprising the neurovascular unit, namely the endothelial cells. Indeed, neuroprotective doses of ALA display vasoactive properties. *Ex vivo*, ALA increased the diameter of the basilar but not carotid artery in mice and rats [53], leading to an approximately 30% increase in artery diameter, which could account for an increase of the CBF observed *in vivo* within 30 min after injection of a neuroprotective dose of ALA [53]. The third mechanistic finding was the ability of ALA to stimulate neuronal plasticity. The reduced long-term mortality rate observed with repeated ALA injections spaced over several days implied additional protective mechanisms other than solely reducing glutamate excitotoxicity within the acute phase of stroke. We have directly investigated whether ALA may stimulate spontaneous biological functions that are implicated in stroke recovery and targeted by restorative therapies. Subchronic ALA treatment induces neurogenesis in the dentate gyrus, identified by an increased number of proliferating immature neurons 3 days after the final ALA injection[58]. Those immature neurons became mature by 21 days. The concept that ALA improved neuronal plasticity was strengthened by the discovery that synaptogenesis was concomitantly stimulated by the ALA treatment, as shown by up-regulation of key proteins involved in synaptic function (synaptophysin-1, VAMP-2, and SNAP-25) as well as proteins supporting glutamatergic neurotransmission (V-GLUT1 and V-GLUT2). In many studies, stimulation of neurogenesis and synaptogenesis implied an up-regulation of neurotrophic factors such as Brain Derived Neurotrophic Factor (BDNF), for which beneficial effects on stroke have been widely described [61]. These changes also correlated with an increase in BDNF protein levels, both *in vivo*, following sub-chronic ALA treatment. Similar results were observed *in vitro* when applying ALA on neural stem cells and hippocampal cultures [58]. With regard to stroke management, the protective actions identified with ALA conform with its role as a

1 treatment for stroke, taking in consideration the necessity of a multimodal action mode on the
2 neurovascular unit by stimulating multiple protective and regenerative mechanisms. A final
3 major mechanistic finding that explains the new concept of nutraceutical being a natural brain
4 preconditioner is the demonstration that ALA is a “natural” preconditioner able to induce
5 delayed cerebral tolerance to ischemia.
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10 **5.5. ALA a nutraceutical stimulating brain preconditioning mechanisms**

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14 The capacity of ALA as a non-ischemic preconditioner able to induce brain tolerance against
15 excitotoxicity-driven neuronal death was demonstrated in rat models of global ischemia and
16 kainic acid-induced epileptic seizures [17]. ALA preconditioning was also successfully
17 demonstrated in a mouse model of transient focal ischemia [58]. The temporal window of
18 brain protection and the protective pathways triggered by ALA preconditioning paralleled
19 preconditioning by sublethal insults and by adenosine and K_{ATP} channel agonists that are
20 acknowledged as gold standards in chemical preconditionings [15, 17, 54]. ALA
21 preconditioning induced the neuroprotective HSP70 heat shock protein and the
22 expression/activation of the transcription factor nuclear factor-kB (NFkB) within a similar
23 time frame and neuronal localization shared by ischemic, epileptic and chemical
24 preconditionings [15, 17, 54]. Such similarities clearly establish ALA as a nutraceutical in its
25 capacity to trigger the ubiquitous pleiotropic protective mechanisms of brain preconditioning.
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38 **6. Conclusion**

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42 This review presents the capacity of alpha-linolenic acid, the precursor of the LC-
43 omega-3 polyunsaturated fatty acid, for protecting the brain from stroke. This review
44 highlights its interest as a supplement to reduce the incidence of stroke and as a nutraceutical
45 to increase brain resistance against stroke damage, in both nutritional and medicinal forms.
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47 The concept underlying the nutraceutical potential of ALA is its pleiotropic targeting of many
48 brain cell types, leading not only to inhibition of the deleterious pathways driven by glutamate
49 excitotoxicity but also to enhance restorative mechanisms such as neurotrophic factor
50 production, neurogenesis and synaptogenesis. The examples of multiple actions of ALA
51 illustrate the current importance of reaching a consensus definition of nutraceutical required
52 for shaping future research in the field. This point seems mandatory because the
53 characterization and use of a nutraceutical like ALA represents a paradigm shift, from
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1 focusing solely on the discovery of new drugs designed for clinical neuroprotection, to the
2 identification of therapeutic capacity of natural food constituents. This is of importance
3 because 1) the medicinal forms of a nutraceuticals may be of advantage as compared to drugs
4 or non-natural preconditioners that display ceiling effects (dose/duration) due to their toxic
5 side effects; 2) development of nutraceuticals against stroke may largely depend not only
6 upon its successful translation to the clinical arena, but also to daily life, notably through
7 functional food development. Such a goal opens an interesting avenue - supported by the
8 characteristics of ALA as a preconditioner - that may be achieved by integrating knowledge
9 drawn from preconditioning research. Though extremely relevant for stroke, this novel
10 concept of nutraceutical preconditioning may not be restricted to ALA or stroke, but may in
11 fact be extend to other existing or novel nutraceuticals and neuropathologies and
12 neurovascular diseases. Hence, the overall effect of ALA, including its capacity to trigger
13 preconditioning, appears to be useful for shaping new recommendations in the nutritional as
14 well as therapeutic approach of stroke, thereby justifying continued investigation in other
15 traumas, epilepsy, SCI and neurodegenerative insults that have been shown to be protected by
16 pre- and post-conditioning.
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8. Conflicts of interest

None

9. Legend of the figures

Figure 1: The different natures of brain preconditioners and their perspectives against stroke

Originally, brain preconditioning refers to an endogenous response to a sublethal stimulus in the brain, which develops tolerance to a subsequent, normally lethal stimulus of the same insult. The protective response to brain preconditioners is pleiotropic in nature. Non-ischemic preconditioners include various sublethal insults, pharmacological/chemical agents and natural compounds including nutrients that are part of our daily diet. The diet aspect provides the rationale of supplementation with a non-ischemic preconditioner that could be a natural product - a nutrient defined as a nutraceutical.

Figure 2: Alpha-Linolenic Acid (ALA) as nutraceutical for stroke: proof of concept

The main distinction between nutraceutical and dietary supplements aimed at supporting the body with the required amount of a certain nutrient required for proper function is that a nutraceutical should have demonstrated a proven efficiency against disease. Alpha-linolenic acid fulfills the restricted definition of the term nutraceutical to compounds isolated or purified from foods, and that efficiency could be evaluated, thereby potentially being prescribed in medicinal forms.

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2 A nutritional approach with an ALA enriched diet from rapeseed oil (left panel) and a
3 medicinal approach with intravenous injection of ALA (right panel) reduce brain damage in a
4 mouse model of ischemic stroke.
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Figure1
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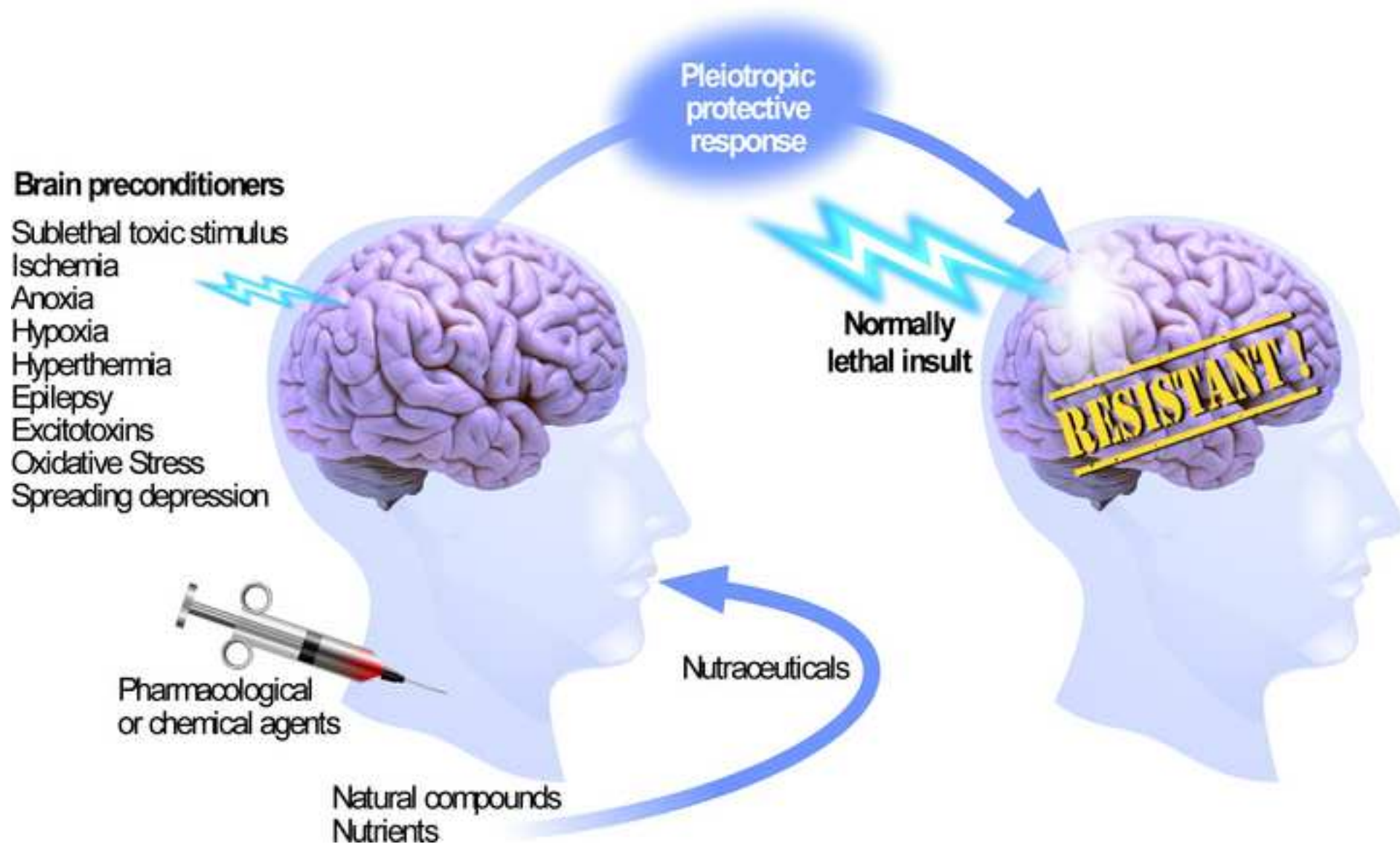
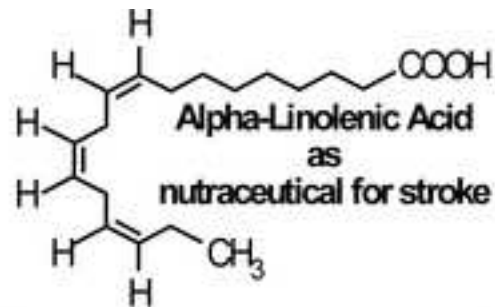
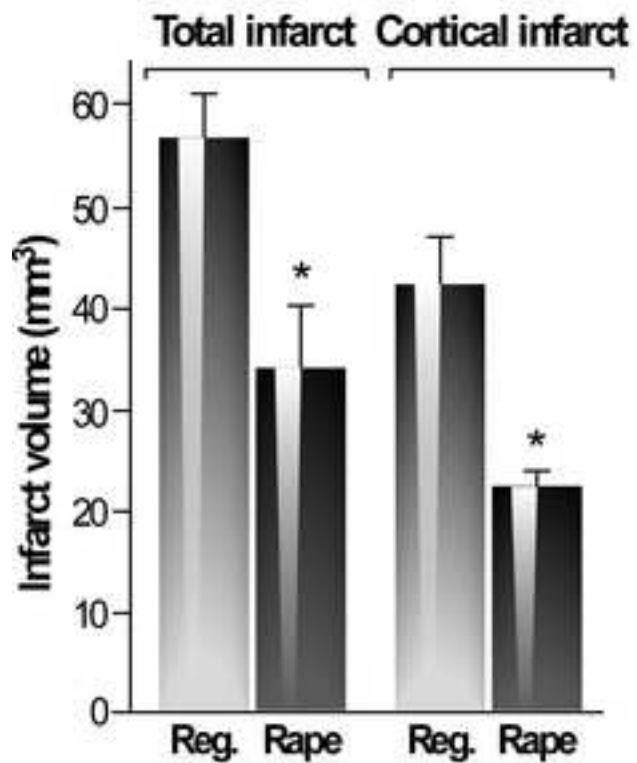


Figure2

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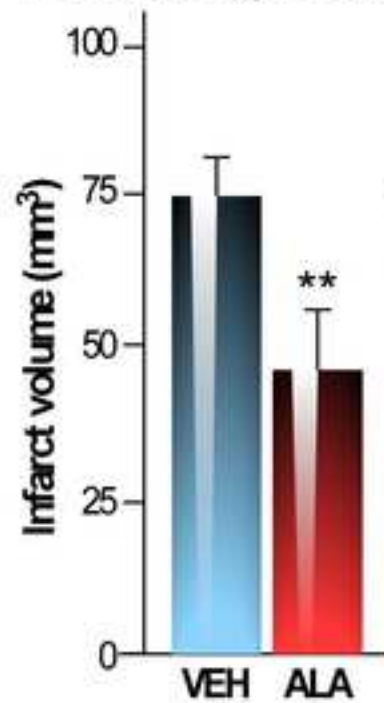


Nutritional approach



Medicinal approach

Preventive injection (i.v.)



Post-treatment (i.v.)

