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Alpha-linolenic acid given as enteral or parenteral nutritional intervention against sensorimotor and cognitive deficits in a mouse model of ischemic stroke

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ABSTRACT

Stroke is a leading cause of disability and death worldwide. Numerous therapeutics applied acutely after stroke have failed to improve long-term clinical outcomes. An emerging direction is nutritional intervention with omega-3 polyunsaturated fatty acids acting as disease-modifying factors and targeting post-stroke disabilities. Our previous studies demonstrated that the omega-3 precursor, alpha-linolenic acid (ALA) administrated by injections or dietary supplementation reduces stroke damage by direct neuroprotection, and triggering brain artery vasodilatation and neuroplasticity. Successful translation of putative therapies will depend on demonstration of robust efficacy on common deficits resulting from stroke like loss of motor control and memory/learning. This study evaluated the value of ALA as adjunctive therapy for stroke recovery by comparing whether oral or intravenous supplementation of ALA best support recovery from ischemia. Motor and cognitive deficits were assessed using rotarod, pole and Morris water maze tests. ALA supplementation in diet was better than intravenous treatment in improving motor coordination, but this improvement was not due to a neuroprotective effect since infarct size was not reduced. Both types of ALA supplementation improved spatial learning and memory after stroke. This cognitive improvement correlated with higher survival of hippocampal neurons. These results support clinical investigation establishing therapeutic plans using ALA supplementation.

1. Introduction

Annually, 15 million people worldwide suffer a stroke. Of these, 30% die and another 30% are left permanently disabled, placing a tremendous burden on family and community. A stroke occurs on average every 40 and 90 s, in the United States and Europe, respectively (Go et al., 2014; Gustavsson et al., 2011). The estimated cost of stroke for 2010 was approximately $74 billion and €64 billion, respectively (Gustavsson et al., 2011; Lloyd-Jones et al., 2010). Apart for thrombolysis – for which inclusion criteria are fairly restrictive - clinical trials for the treatment of acute ischemic stroke have been almost all unsuccessful, leaving patients with an extremely limited repertoire of therapeutic opportunities.

Nevertheless, improvements in population health – particularly in the control of major risk factors of stroke such as hypertension, diabetes, high cholesterol levels and smoking - over the past decades have contributed to reduced stroke mortality. Unfortunately, stroke remains a substantial problem, since mortality represents, at best, a third of annual first-ever strokes. Indeed, in 2007 the

Abbreviations: ALA, alpha-linolenic acid; CA, *Cornu Ammonis*; DAB, 3,3′-diaminobenzidine; DALYs, disability-adjusted life years; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; H/I, hypoxic–ischemic; LC-omega-3, long chain omega-3; MCA, middle cerebral artery; MCAo, middle cerebral artery occlusion; MWM, Morris water-maze; NeuN, neuronal nuclei; NIH, National Institutes of Health; PBS, phosphate-buffered saline; PUFAs, polyunsaturated fatty acids; rTMS, repetitive transcranial magnetic stimulation; SNAP 25, soluble synaptosomal-associated protein of 25 kDa; TPN, total parenteral nutrition; U.S., United States.

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number of stroke survivors was evaluated to be 62 million, and 30–60% of these were estimated to be dependent in some aspect of daily living. These survivors have a high level of disability with more than 50% of patients being left with a residual motor or cognitive/amnesic deficit. Stroke burden was calculated to be approximately 51 million disability-adjusted life years [DALYs] (Johnston et al., 2009; Strong et al., 2007). Therefore, reducing long-term disability by improving recovery would have a substantial impact.

The major stroke-induced impairments are motor and cognitive deficits. Motor impairment is characterized by a loss or limitation of function in muscle control or a limitation in mobility (walking/gaiting) (Jorgensen et al., 1995). In 80% of patients, altered control of the face, arm, and leg of one body side are described as typical symptoms of stroke (Langhorne et al., 2009). Cognitive impairment affects multiple domains, including attention, executive function, visuo-spatial ability, memory and language. As well, focal disorders such as aphasia and neglect are common, as are more diffuse abnormalities such as reduced information processing and executive dysfunction (Cumming et al., 2013).

Confronted with a lack of treatment options, various strategies have been developed to enhance motor recovery, ranging from pharmacological agents prescribed against hypertension, depression or Alzheimer disease, to exercise training (treadmill …) and even to repetitive transcranial magnetic stimulation (rTMS) (Calautti and Baron, 2003; Cramer, 2008; Langhorne et al., 2011). Increasing physical activity and rTMS are also known to improve cognition (Cumming et al., 2013). In cognitive and memory post-stroke rehabilitation, the efficiency of task-specific training remains uncertain mainly due to a paucity of studies (Lincoln et al., 2000; Nair and Lincoln, 2007). Interestingly, most of the motor and cognitive rehabilitation approaches have been shown retrospectively to target neural plasticity and to increase levels of neurotropic factors in the brain (Di Pino et al., 2014; Johansson, 2011).

An emerging preclinical concept to support the recovery of body and brain after stroke is nutrition. Nevertheless how nutrition may affect stroke recovery has not yet been intensely investigated, which is surprising given its great influence as risk factor (Hankey, 2012). Most interventions showing that nutrition could improve the recovery of neurocognitive functions in ischemic stroke patients have been based on protein supplementation provided to counteract stroke-induced protein synthesis depression or malnutrition (Aquilani et al., 2010). Apart from supplementation with vitamins, which may reduce oxidative damage after acute ischemic stroke (B and E groups) or have statin-like effects (Vitamin D) nutritional intervention in stroke patients has been investigated to a limited extent (Pilz et al., 2011).

Severe deficiencies identified in omega-3 polyunsaturated fatty acids (PUFAs), both in the form of alpha-linolenic acid (ALA) and the long chain derivatives eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), stand out as risk factors for cardiovascular and cerebral diseases (Riediger et al., 2009; Kris-Etherton et al., 2002). A body of preclinical studies have documented omega-3 PUFAs supplementation additional benefits in the context of stroke: that as an effective and pleiotropic neuroprotective approach against ischemia (for review (Blondeau and Tauskela, 2013; Gouix et al., 2014; Nguemeni et al., 2013)). As well, fish-oil PUFAs supplemented in diet prior to ischemia was also found exerting a beneficial effect on spatial memory deficits that was not correlated with any benefit in hippocampal neurons survival suggesting that PUFAs may also promote stroke recovery (Plamondon and Roberge, 2008). During the last decade, we identified pleiotropic abilities of ALA to trigger multi-cellular and mechanistically diverse responses, resulting in neuronal protection from stroke (see: (Blondeau, 2016; Blondeau et al., 2015; Blondeau and Tauskela, 2013; Nguemeni et al., 2013)). In a mouse model of stroke, a subchronic post-treatment consisting of three sequential injections of ALA enhanced animal survival rates by three-fold, ten days following ischemia (Blondeau et al., 2009c). Several clinical studies indicated that i.v. perfusion of long chain PUFAs, delivered as parenteral supplementation with a 10% fish oil emulsion (Omegaven, Fresenius-Kabi), may reduce mortality, antibiotic use, and length of hospital stay in different diseases (Heller et al., 2002, 2006). While it seems intuitive that beneficial effects related to fish oil doses may be reliant on the pathology phenotype, it was tempting to speculate some value of omega-3 fatty acid as adjunctive therapy for stroke recovery. ALA supplementation either as an i.v. treatment or as enriched-diet also enhances brain plasticity and improves neurite growth and remodeling in hippocampal neurons (a main site for memory formation) (Blondeau et al., 2009a; Venna et al., 2009), suggesting that ALA may represent an excellent nutraceutical strategy to promote post-stroke recovery.

To provide insight into the potential of ALA on post-stroke rehabilitation, we explored whether ALA supplementation delivered by i.v. subchronic treatment or modification of the daily diet could facilitate recovery of motor and cognitive functions after stroke in the 30 min MCAo model (which has been described as the gold standard for characterization of long-term function in mice (Balkaya et al., 2013)). We first evaluated the effect of the ALA supplementation on the ischemic brain lesion and long-term mortality rate. We further determined motor deficits by the rotarod and pole test and cognitive deficits using the Morris Water-Maze test. Finally, we evaluated the effect of ALA supplementation on selective neuronal lesions that are known to important for stroke rehabilitation.

2. Material and methods

2.1. Animals, diets and treatments

Male 4-week old C57BL/6 J mice (Janvier France Breeding) were housed under standardized conditions and received care according to the policies of European Community Directive 86/609/EEC. The Institutional Animal Care and Use Committees (CIEPAL) approved the study (French Authorization N°06-118). Mice were fed a regular rodent diet (SAFE, France) with an ALA concentration of 0.25% (0.25 g in 100 g of regular diet, a proportion matching the "murine" recommended intake (Bourre et al., 1993; Pudelkewicz et al., 1968)) or an experimental diet enriched in ALA by a factor of three compared to regular chows, for 6 weeks. The content of proteins, minerals, micronutrients, vitamins and the metabolizable energy density provided by the different formulations are as previously described (Nguemeni et al., 2010). Briefly, in the ALA enriched diet (ALA-diet) lipids from rapeseed oil accounts for 10% and ALA for 0.75% by weight. The ALA-diet did not contain any EPA or DHA, in contrast to the regular chow. Body weight, and water and food consumption were monitored through the entire experiments (Nguemeni et al., 2010). With respect to parenteral supplementation (ALA, Enzo Life Sciences), animals were injected in the penil vein 2 h, 3 d, 7 d, 10 d, 14 d, 17 d and 21 d after the occlusion of the middle cerebral artery (MCAo). The timing and dose (500 nmol/kg as a bolus of 50 μl) selected for sequential injections of ALA were based on our previous studies in the mouse model of focal ischemia induced by a longer duration of MCAo (Blondeau et al., 2009c; Heurteaux et al., 2006).

2.2. Middle cerebral artery occlusion

Focal ischemia was induced by a 30 min of transitory MCAo