PUFA-induced neuroprotection against cerebral or spinal cord ischemia *via* the TREK-1 channel

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Abstract: *The nutritional interest of polyunsaturated fatty acids from omega-3, that are mainly present in vegetal and fish oils is now validated by the scientific community. Their beneficial effects have first been reported in coronary heart diseases. Many neurological and chronic diseases are often related to deficiencies in omega-3 and omega-6 and their derivatives. Polyunsaturated fatty acids from omega-3 family are essential to brain growth and cognitive functions. They are recently considered as factors of improvement in some mental diseases. Today, polyunsaturated fatty acids could play a key role in the prevention and/or or the treatment of cerebral diseases. With the development of in vitro and in vivo experimental models, it is now possible to demonstrate the PUFA-induced neuronal protection against major pathologies such as epileptic seizures, cerebral and spinal ischemia. The molecular mechanism of neuronal protection induced by polyunsaturated fatty acids and particularly alpha-linolenic acid is now clarified. The alpha-linolenic target would be a potassium channel, TREK-1, which belongs to the new family of 2-P domain potassium channels (K-2P). The discovery of the physiopathological role of these K-2P channels can represent an important therapeutical challenge not only in cerebrovascular diseases and epilepsy, but also in psychiatry.*

Key words: *polyunsaturated fatty acids, ischemia, epilepsy neuroprotection, K^+ channel*

Introduction

Cerebral ischemia and temporal lobe epilepsy carry a high risk of permanent brain damage mainly due to excitotoxic cell death. These both pathologies have therapeutic and economic considerations, because they affect almost 2% of the intellectual deficits. Cerebral ischemia is the third cause of mortality and the first cause of long term disability. Temporal lobe epilepsy (TLE), characterized by recurrent complex partial seizures (SRS) is one of the most prevalent forms of epilepsy and is frequently associated with pharmacoresistance. During an ischemia or an epilepsy, neurons at risk die as a result of a neurotoxic biochemical cascade initiated by reduced energy stores, membrane depolarisation, excessive neurotransmitter release, accumulation of free fatty acids and lysophospholipids, elevated intracellular calcium, increased oxygen free radicals and neuronal hyperexcitability [1]. The evolution of the major pathophysiological entities of tissue destruction in stroke and epilepsy follows a temporal profile going from minutes-hours to weeks, which corresponds to the acute mechanisms of excitotoxicity and the delayed mechanisms of apoptosis and inflammation leading to the neuronal damage. Since classical therapeutic strategies, that consist in blocking the death pathways were unsuccessful to pass from the bench to the bedside, it can be interesting to test an alternative approach that consists to increase the neuronal resistance by using *the activation of potassium channels* (K^+ channels) to prevent the neuronal hyperexcitability.

K^+ channels are known to be involved in the endogenous regulation of the nervous cell excitability. Opening of K^+ channels may reduce the depolarization triggered by ischemia or epileptic seizure and consequently may reduce brain damage. At a presynaptic level, the K^+ channel activation under physiological conditions will lead to efflux of K^+ resulting in hyperpolarization of the membrane and decrease of the synaptic glutamate release. At a postsynaptic level, activation of the same K^+ channels will prevent some of the postsynaptic effects of glutamate at N-methyl-D aspartate (NMDA) receptors by hyperpolarizing cells and thus favoring blockade by magnesium of NMDA receptor-associated ion channels. Today, more than 77 genes encoding K^+ channels have been identified in the human genome. Recently, a

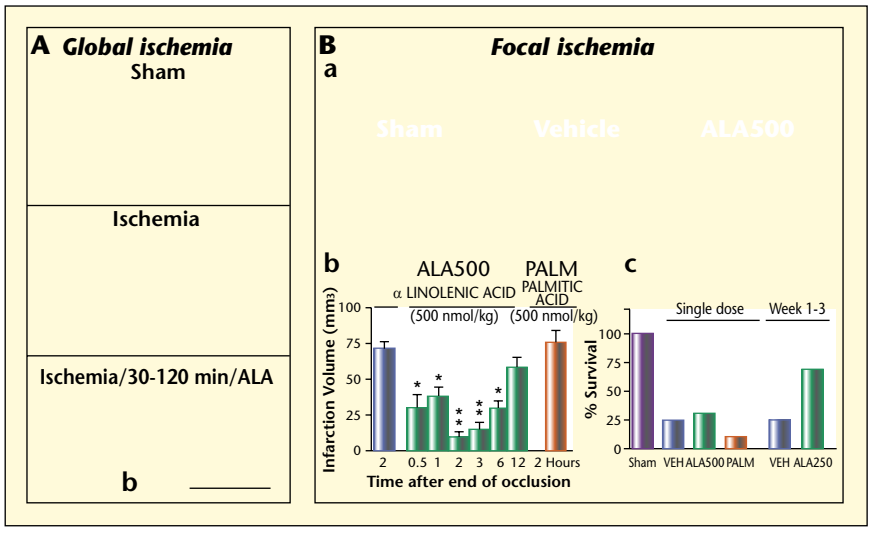

Figure 1. *Alpha-linolenic acid (ALA)-induced neuroprotection in two models of global and focal ischemia. A) Global ischemia (20 min). B) Focal ischemia (1-hour reversible MCAO); a: Neuroprotective effect of a single injection of ALA (500 nmol/kg, iv) on infarct size in TTC-stained brain sections of mice killed 24 h after ischemia; b: Therapeutic window of ALA on infarct volume 24 h after MCAO. c: Comparative effects between three-week therapy and administration of a single dose of ALA on survival rate of mice one month after 1-hour reversible MCAO.*

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newly discovered family of K^+ channels, called *tandem pore domain K^+ channels (K_{2P})*, with four transmembrane-spanning domains and two pore-regions for each protein subunit has been identified [2]. These K_{2P} channels (also called background K^+ channels) and their regulation by membrane-receptor-coupled second messengers, as well as pharmacological agents are therefore important in tuning neuronal resting membrane potential, action potential duration, membrane input resistance and, consequently regulating transmitter release [3]. The class of mammalian K_{2P} channel subunits now includes 15 members. One of them is the TREK-1 channel, which is the most extensively studied [4-6]. The specificity of its regulation is particularly interesting in relation with neuronal disease states. Mechano-gated and arachidonic acid-activated TWIK-related K^+1 (TREK-1), highly expressed in the brain [7] is a signal integrator responding to a wide range of physiological and pathological inputs. It can be activated by physical stimuli such as stretch, depolarization, intracellular acidosis and warm temperature. In relation with neuroprotection, TREK-1 is upmodulated by volatile anaesthetics, riluzole [8] (a well-known neuroprotective agent) [9-12] and also with lysophospholipids and polyunsaturated fatty acids (PUFA) including arachidonic acid (AA), docosahexaenoic acid (DHA) and alpha-linolenic acid (ALA) [6, 13].

PUFA and neuronal protection

Using *in vivo* models of ischemia, our laboratory has shown that PUFAs are able to induce a strong neuronal protection against the deleterious effects of cerebral [14, 15] and spinal [16] ischemia. In the model of global ischemia [14], induced in rats by cauterization of vertebral arteries and transient (20 min) clamping of both carotids, an intravenous injection of ALA at a dose of 500 nmoles/kg 30 min up to 2 hours post-ischemia strongly reduces the neuronal loss of CA1 pyramidal cell layer induced by severe ischemia and blocks apoptosis revealed by TUNEL assay *(figure 1)*. The transient (60 min) occlusion of middle cerebral artery (focal ischemia) induces in mice focal cortical and subcortical lesions and reproduces human clinical observations of a stroke. The quantitation of infarct volume at 24-hour postischemia shows that an injection of ALA, but not palmitic acid, a saturated fatty acid reduces the infarction volume with a therapeutic window from 30 min to 6 hours postischemia [15]. Interestingly, at one month after reperfusion, the best protection is obtained with a three-week therapy of ALA (250 nmoles/kg) with a 70% survival rate *(figure 1)*. Spinal ischemia is a devastating complication of thoracic and thoracoabdominal aortic surgery, which induces a severe and often definitive paraplegia. In the model of spinal ischemia [16], rats are submitted to cross-clamping of the aortic arch and left subclavian artery for 15 min. The rats treated with 200 nmol/kg of ALA at the onset of reperfusion have a better neurologic function *(figure 2)*. PUFA are also able to induce a brain tolerance [17]. Cerebral preconditioning is a powerful endogenous protective mechanism in which moderate ischemic or epileptic insult

A ***Spinal ischemia***

Neurological status 24 h after ischemia (40 min)

Tarlov Score	Vehicle	ALA (200 nmole/kg) onset of reperfusion
No movement	10	-
Slight movement	-	1
Sits with assistance	-	-
Sits alone	-	-
Weak hop	-	1
Normal hop	-	8

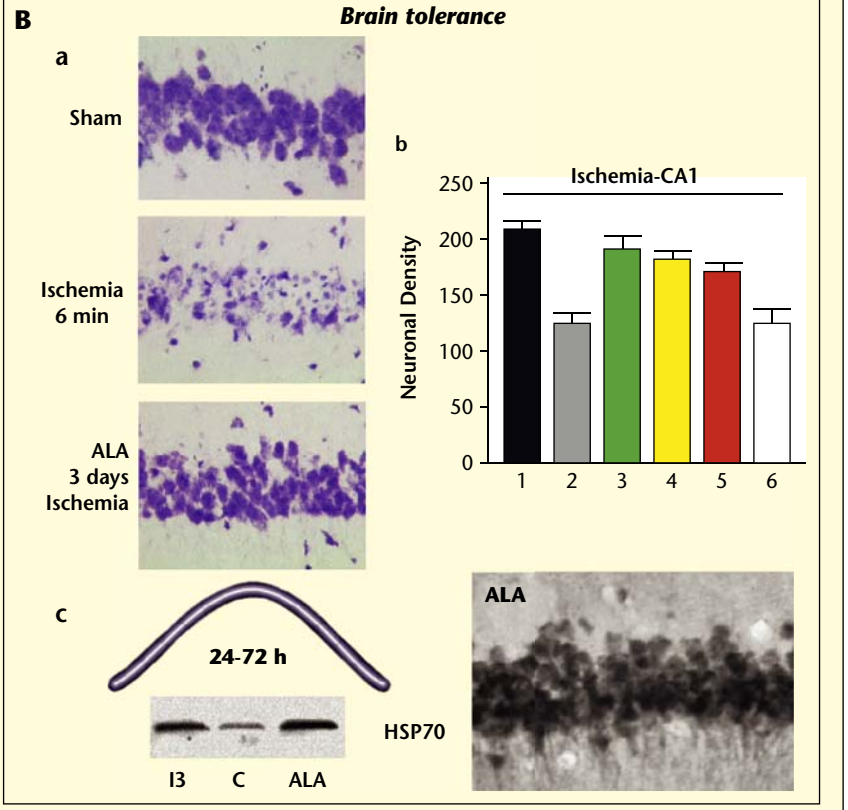

Figure 2. *ALA-induced neuroprotection in a model of spinal ischemia (40 min) and in brain tolerance. A) Neurologic score (Tarlov) after 24 hours of reperfusion. B) Ischemic tolerance induced by a ALA-pretreatment (500 nmol/kg 3 days before 6 min global ischemia). a: Representative photographs of hippocampal CA1 region 7 days postischemia. b: Neuroprotective effects of different PUFA pre-treatment on the neuronal density in CA1 region 7 days postischemia. 1. Sham, 2: Vehicle, 3: DHA, 4: ALA, 5: AA, 6: PALM. C: Western blot and immunochemistry showing the induction of resistance heat shock protein HSP70 in the protection window (24-72 hours) induced by ALA pre-treatment (500 nmol/kg), or ischemic preconditioning (3 min) in CA1 substructure.*

provide a neuroprotective adaptation of the brain against subsequent severe ischemic or epileptic insult, normally lethal for neurons [18]. Because ATP-sensitive potassium channels opening through adenosine A_1 receptor activation are a central early step in cerebral preconditioning, it is possible to mimic preconditioning pharmacologically with adenosine agonists and K_{ATP} openers [19, 20]. Similarly, an intravenous injection of ALA at 500 nanomoles/kg induce a potent brain tolerance when it is administered as early as 3 days before severe ischemic or epileptic injury [21] *(figure 2)*. Palmitic acid, which does not activate TREK-1 channels fails to protect the brain in both pathologies. The potent delayed neuroprotection induced by ischemic, epileptic or pharmacological preconditioning requires *de novo* synthesis of proteins including manganese superoxide dismutase, Bcl_2 and heat shock protein 70 in the time window of protection (1 to 3 days). ALA-induced preconditioning induces a strong HSP70 expression in the cerebral structures including the CA1 region of hippocampus, normally damaged by severe ischemia [17] *(figure 2)*.

TREK-1 channel, lipids and neuronal protection

The TREK-1 channels have no specific blockers. In order to study the physiopathological role of TREK-1 *in vivo*, its gene has been disrupted by homologous recombination in the mouse. Using this knockout (KO) mouse model, recent studies indicate a central role for TREK-1 in general anaesthesia, pain perception, depression and neuroprotection [13, 22-24]. To test the resistance of KO mice to global ischemia, a 30 min transient bilateral occlusion of common carotid arteries (CCA) is associated with a systemic hypotension (Mean Arterial Blood Pressure, MABP 30 ± 3 mmHg). During the recovery period, most of the knockout mice developed seizures of progressive severity leading to a 40% increase in the number of deaths for the *Trek1*$^{-/-}$ mice compared to the *Trek1*$^{+/+}$ mice [22] *(figure 3)*. Using two models of epilepsy (seizures induced by epileptogenic doses of kainate (a glutamate agonist, 22 mg/kg) or pentylenetetrazole (a GABA antagonist, 40 to 55 mg/kg), results show that *Trek1*$^{-/-}$ mice were much more sensitive to epilepsy. More than 75% of the mutant mice died within 3 days of kainate administration, compared with 3% of *Trek1*$^{+/+}$ mice, and the average maximum intensity of seizures observed in *Trek1*$^{-/-}$ mice increased by 33%. *Trek1*$^{-/-}$ mice developed generalized convulsive seizures with the appearance of bilateral spike-wave discharges with spike frequencies and amplitudes higher than in *Trek1*$^{+/+}$ mice. Activation of *c-fos*, routinely used as a biochemical marker of neuronal excitability is drastically enhanced in *Trek1*$^{-/-}$ mice compared to *Trek1*$^{+/+}$ mice, particularly in CA3 subfield at 120 min after kainate injection [22]. While ALA treatment is neuroprotective against global ischemia and seizures, such neuroprotection is lost in Trek1$^{-/-}$ mice, which indicates that protection by PUFA is mediated by TREK-1 opening [13, 22] *(figure 3)*.

Conclusion

It has been well established that PUFA and particularly ALA and DHA administered in acute treatment are potent neuroprotectors against ischemia and epilepsy. In term of prevention, PUFA induce a strong ischemic and epileptic tolerance. *Trek1* knockout mice provide evidence for the important functional role

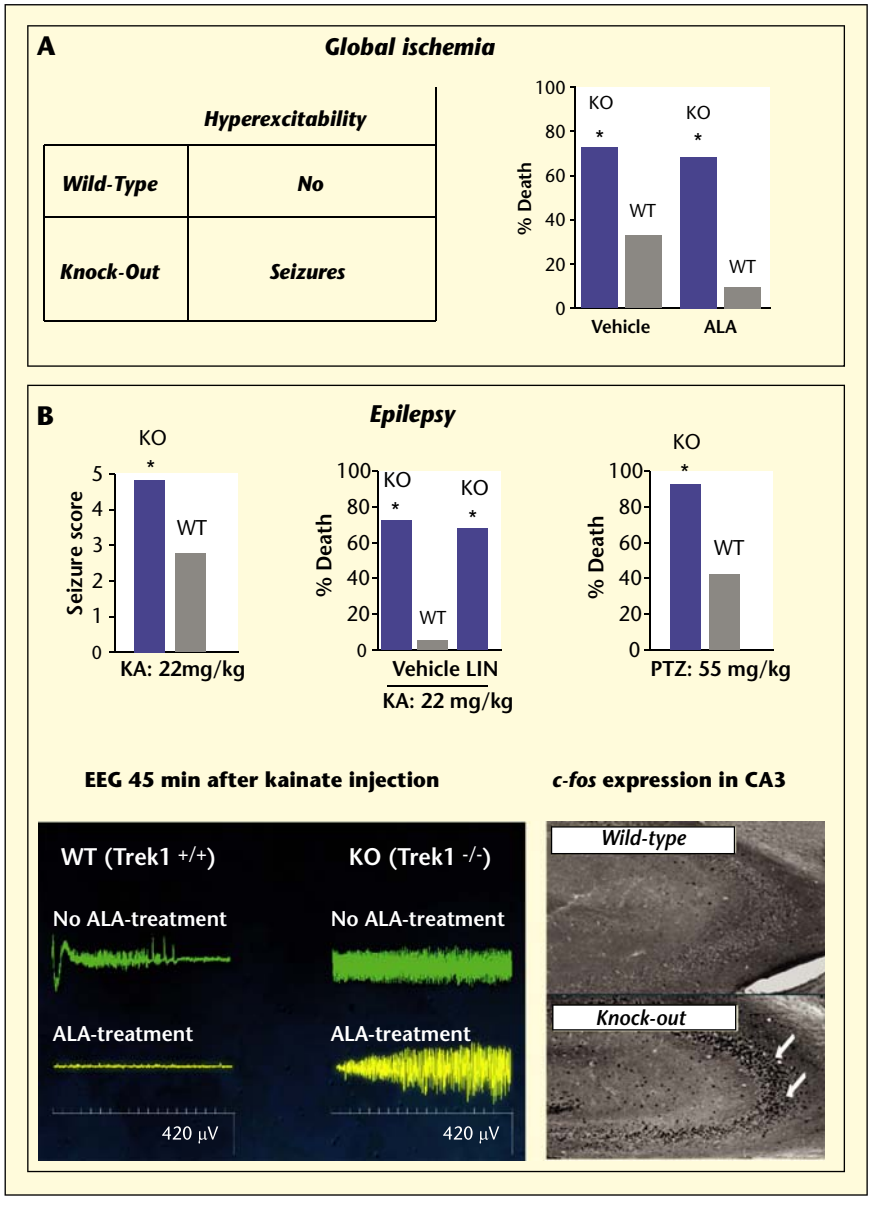

Figure 3. *The TREK-1 channel is involved in the neuroprotection induced by PUFA. A) Increased vulnerability of TREK-1-deficient mice to global ischemia and loss of the neuroprotective effect of ALA (500 nmol/kg) in Trek1*$^{-/-}$ *mice. B) Increased susceptibility to epileptic agents in TREK-1-deficient mice. Seizure behavior and mortality rate are analyzed in wild-type and mutant TREK-1 mice after KA or PTZ injection. Seizures were scored for 2 h after intraperitoneal injection with KA (22 mg/kg) or PTZ (55 mg/kg). EEG following KA (22 mg/kg) shows the increased KA susceptibility of mutant mice as compared to wild-type mice and the lost of ALA protection against KA-indued seizures. There is an Increased expression of c-fos protein in CA3 pyramidal neurons in mutant mice 120 min after KA treatment.*

of this K^+ channel in PUFA-neuroprotection. At the pharmacological level, future studies will be needed to identify high-affinity openers of the TREK-1 channel that might prove useful for the treatment of a range of neuronal disease states.

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