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Review

Excitotoxicity, neuroinflammation and oxidant stress as molecular bases of epileptogenesis and epilepsy-derived neurodegeneration: The role of vitamin E

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ABSTRACT

Glutamate-mediated excitotoxicity, neuroinflammation, and oxidative stress are common underlying events in neurodegeneration. This pathogenic "triad" characterizes the neurobiology of epilepsy, leading to seizure-induced cell death, increased susceptibility to neuronal synchronization and network alterations. Along with other maladaptive changes, these events pave the way to spontaneous recurrent seizures and progressive degeneration of the interested brain areas.

In vivo models of epilepsy are available to explore such epileptogenic mechanisms, also assessing the efficacy of chemoprevention and therapy strategies at the pre-clinical level. The kainic acid model of pharmacological excitotoxicity and epileptogenesis is one of the most investigated mimicking the chronicization profile of temporal lobe epilepsy in humans. Its pathogenic cues include inflammatory and neuronal death pathway activation, mitochondrial disturbances and lipid peroxidation of several regions of the brain, the most vulnerable being the hippocampus. The importance of neuroinflammation and lipid peroxidation as underlying molecular events of brain damage was demonstrated in this model by the possibility to counteract the related maladaptive morphological and functional changes of this organ with vitamin E, the main fat-soluble cellular antioxidant and "conditional" co-factor of enzymatic pathways involved in polyunsaturated lipid metabolism and inflammatory signaling.

The present review paper provides an overview of the literature supporting the potential for a timely intervention with vitamin E therapy in clinical management of seizures and epileptogenic processes associated with excitotoxicity, neuroinflammation and lipid peroxidation, i.e. the pathogenic "triad".

1. Introduction

Epilepsy is a frequent neurological disease affecting approximately 1% of the world's population [1]. Additionally, a greater percentage of people experiences seizure at least once in their life [2], thus resulting in significant social and demanding public health issue with a marked impact on quality of life.

Seizure episodes have been defined as "transient symptoms and/or signs due to abnormal and simultaneous neuronal activity of a population of neuronal cells in the brain" [3]. These episodes can be triggered by almost any insult that alters brain function, due to acquired causes (such as stroke or traumatic brain injury), infectious diseases, autoimmune diseases, and genetic mutations; but, in some patients the causes of seizures remain unknown [4]. According with the International League Against Epilepsy 2017 basic seizure classification, seizures can be classified as focal, generalized or unknown onset [5]. It is worth specifying that epilepsy, as a brain disease, can be defined after any of the following conditions: "(1) at least two unprovoked seizures occurring more than 24h apart; (2) one unprovoked seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years; (3) diagnosis of an epilepsy syndrome" [6]. This would mean that

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"epilepsy" is a condition that develops if spontaneous seizures arise repeatedly. The process inducing epilepsy to develop is called "epileptogenesis" which takes from months to years in humans and involves a number of structural and functional mechanisms inducing maladaptive neural plasticity and converting normal brain into a state with a propensity for generating seizures [6]. According to this definition, epileptogenesis results to be a "gestational" period needed overt epilepsy disease to occur [7,8]. How an initial brain insult elicits epilepsy is still a matter of debate, however, glutamate excitotoxicity, neuroinflammation, and oxidative stress appear to represent a pathogenic "triad" which characterizes the neurobiology of different brain disorders, including epilepsy, leading to seizure-induced cell death, increased susceptibility to neuronal synchronization and network alterations.

The in-depth understanding of epileptogenic mechanisms would allow designing antiepileptogenic strategies addressed to impede overt epilepsy to occur, and to this aim, animal model approaches are instrumental [9]. Consistently, epileptogenesis models induced by lesions, such as chemoconvulsant insults, kindling, and traumatic brain injury, have become available to study distinct aspects of the human focal epilepsy pathology. In particular, considering the major incidence of temporal lobe epilepsy (TLE) in human, chemoconvulsant insult models, which reflects specific features of this disease by affecting hippocampus formation, provide opportunities to overcome the lack of human samples and gain insights into early stages of epileptogenesis [10,11].

Since the brain is highly susceptible to oxidative stress due to its low antioxidant enzyme activity, and considering that a wealth of studies have indicated that free radicals can act as a pathogen in the epilepsy disease [12,13], natural compounds with antioxidant properties have been evaluated over time in order to find out a therapeutic strategy for preventing the development of epilepsy in patients at risk, by lowering seizure-triggered maladaptive neural plasticity underlying epileptogenic processes [14,15]. In this context, clinical evidence from one study published in 1984 by Kovalenko and colleagues [16] revealed the capability of α-tocopherol (α-T), the vitamin E isoform with the highest in vivo biological activity and bioavailability [17], to improve seizure control in drug-resistant patients. The study recruited seventeen patients suffering from various forms of epilepsy and exhibiting a resistance to the anticonvulsant and psychotropic therapies; they received α-T treatment throughout a month after which the following beneficial effects were observed: 1. improvement of electroencephalogram (EEG) outcome in terms of reduction of pathological signs; 2. significant decrease of seizure frequency, up to be completely arrested in four patients; 3. abatement of lipid peroxidation product plasma levels. These findings were confirmed in a more recent paper published by Mehvari and coworkers [18]. These Authors tested again the influence of vitamin E on seizure control and redox state in sixty-five patients with epilepsy under chronic antiepileptic intake. The results they have obtained highlighted that vitamin E produced a significant decrease in seizure frequency and improved EEG, and these effects in most patients were associated with a better antioxidant status. On the other hand, it is proper also to mention the results obtained by Raju and coworkers in a double-blind, cross-over trial [19], where they did not find significant improvement in seizure control in adolescent and adults affecting by refractory epilepsy under vitamin E treatment, as referred in [20]. Differences in antiepileptic drugs given to patients and in vitamin E dosage, timing and treatment duration might account for the inconsistencies observed across the studies.

In keeping with beneficial effects of vitamin E, findings obtained in animal models pointed out that vitamin E (as α-T) pre-treatment attenuates convulsive behavior and brain oxidative stress [21–25], thereby decreasing the severity of seizures and their detrimental effects on brain tissue and functions. Altogether, these findings suggest the possibility to intervene with α-T on mechanisms prompting epileptogenesis and the consequent defect of redox homeostasis in different areas of the brain.

Among the animal models of epilepsy available to explore epileptogenic mechanisms, kainate (KA) status epilepticus is widely chosen because it mimics the TLE human disease [26], thus representing a validated animal model to study the onset and development of chronic epilepsy (recently reviewed in [27]). Pathogenic cues typical of this animal model of epilepsy include inflammatory and neuronal death pathway activation, mitochondrial disturbances and lipid peroxidation occurring in several regions of the brain, the most vulnerable being the hippocampus. Through the use of this animal model our group has confirmed the role of excitotoxicity, neuroinflammation and lipid peroxidation as underlying molecular events of epileptogenesis [28,29]. In these studies, the possibility to counteract these events with α-T was demonstrated, thus reducing the related maladaptive changes of the brain and the hippocampal neuronal network excitability.

Advisedly, these effects of vitamin E were initially ascribed to its antioxidant properties, considering that oxidant stress is one of the underlying events in excessive neuronal discharge and an instigator of increased seizure susceptibility [30,31]. However, recent in vitro and in vivo studies revealed effects by this vitamin independent from its antioxidant function (reviewed elsewhere in [32,33] and references therein) that may also affect some oxidant stress-independent pathogenic factors of epilepsy. These include the modulation of enzymes, signal transduction pathways and transcription factors associated with the inflammatory response (recently reviewed in [17,34]), such as the nuclear factor kappa-light-chain-enhancer (NFkB) of activated mononuclear cells, and the lipoxygenase (LOX)-dependent and cyclooxygenase (COX)-dependent pathways of arachidonic acid oxidation to generate inflammatory and vasoactive eicosanoids.

Neuroinflammation is a main player in the pathophysiology of epilepsy, since neuroglial activation and cytokine production heightened seizure-induced neurotoxicity, contributing to epileptogenesis [35,36]. Actually, the expression of inflammatory mediators, such as interleukin-1 β (IL-1 β) and tumor necrosis factor-α (TNF-α), are induced by seizure activity in astrocytes and microglia, originating a cascade of downstream inflammatory events [37–40] which results in the alteration of neuronal excitability increasing the probability of seizure production [41,42]. Moreover, new perspectives for a role of vitamin E therapy in neuroinflammatory disorders have recently emerged by the investigation of enzymatic metabolites of this vitamin [17,43]. Some of these metabolites are potent immunomodulation and lipid signaling molecules involved in inflammatory and degenerative processes of tissues. Their investigation may thus hold great potential in the chemoprevention and therapy of seizure-induced brain damage and epileptogenesis.

In this view, the present review focuses on the molecular bases of lipid oxidation and inflammatory cues of epilepsy condition thus examining the evidence supporting the potential of a timely intervention with vitamin E in the clinical management of seizures and epileptogenesis, based on the abatement of excitotoxicity, neuroinflammation and oxidative stress.

2. Vitamin E and excitotoxicity

Excitotoxicity induced by glutamate pathway persistent discharges has convincingly been proposed as a mechanism for epilepsy to develop [44] and to eventually persist as a chronic form of neurodegeneration. In the brain, glutamate is the major excitatory neurotransmitter and its excessive release leads to repeated depolarization-repolarization cycles in glutamate terminals. As a consequence, the degeneration of postsynaptic neurons takes place due to the increase in calcium influx, mainly through NMDA (*N*-methyl-D-aspartate) ionotropic receptor activation [45–47]. Consistently, micro-dialysis studies have shown elevated levels of glutamate in the human brain and animal models of epilepsy, causative of an association between the prolonged convulsive activity and duration of the epileptic episode [48]. In addition, NMDA

receptor activation has been found out to trigger NADPH oxidase 2 (NOX2) assembly and activity, which has been shown to produce reactive oxygen species (ROS) during seizures [49].

Different astrocyte and neuron enzymes are involved in glutamate metabolism [50]. Astrocytes normally take up glutamate released from synapses and quickly convert it to glutamine, a non-excitotoxic amino acid, by glutamine synthase (GS) [51]; glutamine is then transferred back to the neurons where it is used for the synthesis of neuronal glutamate, via the glutamine-glutamate cycle pathway, and for the synthesis of GABA, via the glutamine-glutamate-GABA cycle pathway. In this view, GS emerges as a pivotal enzyme in glutamate metabolic pathways and its defect was identified in astrocytes of epileptogenic hippocampal formation from a subset of patients with temporal lobe epilepsy (TLE) [52], consistent with increased extracellular concentrations of glutamate and decreased cellular content of glutamine [53]. In keeping with these findings, a number of reports have pointed to GS as a key pathogenic target for epileptic seizures [52]. Accordingly, astrocytes GS expression is down-regulated following status epilepticus (SE) [52] and enzyme activity is directly inhibited by the seizure-induced generation of ROS [54]. Thus, the reduced expression and activity of this enzyme in hippocampal astrocytes would decrease glutamate clearance, leading on one side to an abnormal build-up of the extracellular excitatory neurotransmitter glutamic acid, and on the other side to a reduced synthesis of glutamine to be transformed in GABA inhibitory neurotransmitter [55], paving the way to further seizure activity. Indeed, the occurrence of recurrent seizures is commonly believed to be closely related with an imbalance in the excitatory and inhibitory drive, mainly due to reduced efficacy of GABAergic inhibition [56]; for this reason, GABA receptors are considered the main target of current and future antiepileptic drugs. However, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors have also been recognized as pivotal mediators in epileptogenesis, and their antagonists are a therapeutic option now under investigation by different groups [57,58]. Recently, a different point of view regarding this issue has come from Elahian and coworkers [59], which suggested that low-voltage fast focal seizures may occur from an enhanced inhibitory neuron firing followed by an increase in excitatory neuron firing, leading to a distinct pattern of inhibitory/excitatory imbalance.

2.1. Vitamin E and glutamate/GABA balancing: impact on seizure frequency and epileptogenesis

Glutamine synthase could represent another eligible pharmacological target, besides GABA and glutamate receptors, in the therapy of the aforementioned disruption of glutamate/GABA balancing and persistent excitotoxicity of SE. In line with this, some pathological states involving a rise in either ammonia or glutamate might benefit from GS regulation [60,61].

According to a role of astrocyte GS deficiency in the epileptogenic phenotype of human TLE [52], a marked decrease of GS enzyme expression in hippocampal tissue was also observed in the KA-induced seizure model of the rat [62–64]. Besides to GS deficiency, this model reflects several of the neuropathological features of human TLE being characterized by a state of prolonged SE followed by spontaneous recurrent seizures beginning after a latency of about 15 days. Surrogate indicators of brain oxidant stress were increased following overt SE, including lipid peroxides and lipoxidation-derived protein carbonyls [28,29,65] (discussed later).

In this model of epilepsy, we found that pharmacological doses of α-T (250 mg/Kg b.w. via intraperitoneal - i.p. - for 4 days) administered immediately after the appearance of overt SE, counteracted the GS decline [28] realistically promoting the restoration of glutamate metabolic pathways and, consequently, of the glutamate and GABA hippocampus content, thus contributing to reduce excitotoxicity. The mechanism(s) by which α-T can almost completely restore such KA-induced defect of GS expression remain(s) elusive and is currently under investigation. In principle, however, lowering of oxidative stress by α-T antioxidant property may provide a straightforward mechanism to avoid free radical-mediated GS damage and reduced activity [54]. Natural compounds with antioxidant properties have been found regulating GS activity by preventing oxidative stress [66] that is known to inhibit this enzyme [54]. Notwithstanding, antioxidant-independent mechanisms may also have a role in mediating the reported in vivo effect of α-T on GS expression/activity of the KA model. In keeping with this, α-T can inhibit protein kinase C δ (PKCδ) activity in rat hippocampus [67], and PKCδ was found to exert an inhibitory effect on GS expression [68]. Accordingly, generalized PKC activation was shown to induce seizure discharges [69].

On the other hand, it can be hypothesized that α-T may affect expression and/or activity of GABA and/or AMPA receptors. It is known that epileptic hippocampus shows $GABA_A$ receptors ($GABA_AR$) which become less responsive to repeated activation (desensitization) in comparison to normal tissue both in humans and in animal models of epilepsy [70–74]; this behavior named as $GABA_AR$ rundown, may represent a key mechanism in disrupting inhibitory function, thus contributing to excitation-inhibition imbalance [75]. Taking advantage of the technique of membrane microtransplantation in *Xenopus* oocytes, we tested the effect of α-T treatment (250 mg/Kg b.w. via i.p. for 4 days, followed by 2 mg/Kg b.w. up to animal killing at 15 days from SE) on electrophysiological properties of $GABA_A$ and we did not find any significant effect of α-T on the degree of current desensitization, and on GABA current amplitude and chloride homeostasis as well [29]. AMPA expression, channel subunit composition and functioning, and AMPA/GABA current ratio were also unaffected. Altogether, these findings do not support a direct effect of α-T on GABA and AMPA receptors.

However, indirect effects of α-T on these receptors cannot be ruled out. It has to take into account that epileptic seizure can disrupt glutamate/GABA homeostasis by inflammatory pathway activation [76]; cytokines released from activated microglia are reported to influence glutamate receptors and transporters in astrocytes, thus influencing neuronal excitability [76]. As described below, α-T is able to counteract microglia activation, quenching inflammatory routes that may indirectly contribute to perturb the glutamate/GABA balancing. An excess of reactive species produced during the inflammatory process and the resulting damage to membrane lipids and proteins are other detrimental factors for this balancing that could be prevented with α-T.

3. Vitamin E and neuroinflammation

The interaction between the innate immune system and injured brain tissue is known as neuroinflammation, a complex process that involves the release of inflammatory molecules mainly by microglia and astrocytes [77]. Increasing evidence points at the pathogenic role of neuroinflammation in epilepsy (e.g. [77–80]), being neuronal activity related to epileptic seizures among the factors able to trigger an inflammatory response in the brain [78]. In principle, “neurogenic neuroinflammation” would represent an adaptive response allowing the brain to cope the enhanced metabolic demand occurring during seizures, but it turns to maladaptive response when it is intense and persistent [81] spreading to remote sites. Indeed, the broad number of inflammatory mediators released during and after seizures promote the activation of inflammatory pathways in neurons, glia and in blood-brain barrier, and can alter neuronal excitability and synchronicity, by modulating receptor function and expression, thus contributing to epileptogenesis [82–85]. Therefore, quenching neuroinflammation by targeting molecular mediators and their signaling pathways would represent an important therapeutic strategy to attaining antiepileptogenic effects.

3.1. Vitamin E-dependent inhibition of the neuroinflammatory process: mechanistic aspects

Over the last decades, in vitro and in vivo evidence has been obtained on the role of vitamin E as an inhibitor of neuroinflammatory responses and glial cell reactivity upon exposure to various types of insults [86–91], such as ischemia-reperfusion and exposure to the bacterial wall component lipopolysaccharide, or in animal models of Alzheimer's disease. No one study to our knowledge has investigated this role of vitamin E in epilepsy so far. In an attempt to fill this gap, we have performed a series of studies on neuroinflammation and glial activation cues in the rat hippocampus after treatment with the proconvulsant agent KA [28,29,65]. In these studies, vitamin E was observed to have positive effects on the investigation endpoints (that included microglial activation, reactive astrocytosis and cytokine expression) independent of its administration procedure. More in detail, three alternative protocols were examined:

i) treatment with α-T before inducing SE; the vitamin was administered with the animal diet (RRR- α-T form, 10 g/Kg pellet) [65];
ii) post-ictal treatment with intraperitoneal injection of pharmacological doses of α-T (250 mg/kg b.w.) starting soon after SE and lasting four days [28]; this time point was chosen because it corresponds to the beginning of a transition phase (spanning between 3 and 7 days after SE) in which, although in absence of EEG and behavioral seizures, specific neuroinflammatory responses are still active as an underlying event in the onset of chronic epilepsy [37,92];
iii) post-ictal treatment with intraperitoneal injections of pharmacological doses of α-T soon after SE and lasting fifteen days (250 mg/kg b.w. for the first 4 days followed by 2 mg/kg for the remaining days) [29], a gap corresponding to the latent period duration in this epileptic rat model, at the end of which spontaneous recurrent seizures start to appear [62–64].

In each of these experimental conditions, α-T treatment lowered the hippocampal expression of the glial and microglial markers glial fibrillary acidic protein (GFAP), major histocompatibility complex (MHC), ionized calcium-binding adapter molecule 1(IBA-1), and that of the (early) pro-inflammatory cytokines IL-1β and TNF-α, proofing a significant breaking of brain inflammatory pathways that were activated during the pharmacological epilepsy protocol. Noteworthy, protein levels of IL-6 remained up-regulated by α-T treatment until fifteen days after SE; in principle, this increase of IL-6 expression could appear inconsistent with its well-known role as inflammatory cytokine (for a review see [93], but following the overt SE, IL-6 expression time-course differed from that of the other inflammatory cytokines, reaching maximal levels within 24 h and then decreasing after 3 days [94,95]. In addition, it has to be considered that IL-6 shows a multifunctional nature spanning from pro- to anti-inflammatory activity and including effects on metabolic control and repair of the inflammatory lesion [96]. The importance of these functions in homeostatic compensations of epileptic foci, and in general of the brain, can easily be foreseen; IL-6 has been proposed to contribute to neuroprotection after SE [97], and the lack of IL-6 production is associated with higher seizure susceptibility to some chemoconvulsants [98]. Even though the exact mechanisms underlying the neuroprotective function of IL-6 have not been fully elucidated, its ability in down-regulating the synthesis of pro-inflammatory cytokines IL-1β and TNF-α has been demonstrated [99,100]. In keeping with this, the lowered protein expression of IL-1β and TNF-α in epileptic rats treated with α-T observed in our studies could be ascribed, at least in part, to upregulation and immunomodulatory effect of IL-6.

It is also relevant to consider that cytokine expression and signaling, along with many other pathophysiological processes, depend on the gene-modulation effect of epigenetic factors. These include small non-coding RNAs also named microRNA (miRNAs) [101,102]. Several studies underpin the theory that miRNAs are implicated in the pathogenesis of epilepsies and miRNA dysregulation has been abundantly documented after experimentally-induced SE and in human brain with epilepsy condition as well [103–105]. In this context, miR-146a takes a prominent role as a crucial mediator of the neuroinflammatory response; consistent with the finding obtained in human TLE, animal models showed an up-regulation of this miR occurring in the latent period of epileptic disease [106]. Accordingly, pro-inflammatory cytokines, mainly IL-1β [102,107], were found to induce miR-146a expression, which appears to act in a “negative regulatory loop” [108] to control the inflammatory response. In this scenario, miR-146a expression was significantly increased in the rat model of KA epilepsy, and α-T treatment was able to hold back to control levels such miR-146a up-regulation. The time course of these changes in miR-146a during the protocols of pharmacological SE and vitamin E therapy may lead to hypothesize the following mechanism: α-T drops down the expression levels of IL-1β and TNF-α pro-inflammatory cytokines, and it is able to prevent miR-146a up-regulation induced by SE. The latter may increase IL-6 release, thus contributing to further down-regulate IL-1β and TNF-α protein levels with the restoration of morphofunctional and behavioral aspects. However, in an attempt to provide a mechanistic explanation about the action of α-T on neuroinflammation reduction, it has to be also considered the possibility that this molecule might exert a direct effect on miR-146a expression as its down-regulation has been detected in α-T-treated animals not exposed to KA [29]; this is in line with findings that showed the ability of α-T in down-regulating miR-125b, another epigenetic player of the inflammatory response [109].

Finally, it is worthy to mention that alterations in hippocampal levels of miR-146a by SE and α-T treatment during the latent period were also recapitulated in the serum of the same animals [29], supporting the clinical relevance of miRNAs as diagnostic and prognostic biomarkers of human diseases [110], epilepsy is included.

3.2. Blood-brain barrier disruption

Another crucial element that contributes to epileptogenesis is a dysfunctional blood-brain barrier (BBB). Seizure activity has been suggested to cause this type of dysfunction [111] together with systemic factors, such as the release of immune mediators that may increase endothelial permeability [111]. Indeed, SE-induced pro-inflammatory cytokine surge is closely related with BBB breakdown [112] and CNS permeation of blood-borne entities ranging from small molecules to cells [113,114]. Furthermore, BBB integrity in epilepsy is also affected by vascular endothelial growth factor (VEGF), a potent modulator of endothelial permeability released by neurons in response to seizures. This factor triggers vascular remodeling and angiogenesis that sustain the formation of new leaky blood vessels [115,116].

The presence of serum proteins in the brain is one of the consequences of BBB disruption; among these proteins, albumin has been demonstrated to be taken up or bound to neurons, astrocytes and microglial cells after SE [114], and to influence neuron excitability and inflammation. The extravasation of albumin due to damaged BBB and the consequent uptake of this protein by astrocytes activate transforming growth factor β (TGFβ)-mediated signal transduction [117]; this pathway influences astrocyte properties decreasing Kir 4.1 potassium channels. The consequent increase of extracellular potassium concentration depolarizes neurons enhancing their firing [118]. In addition, it has been proven that albumin uptake in neurons increases the glutamate release [119], further enhancing neuronal excitability. Extravasated albumin also contributes to brain inflammation through transcriptional up-regulation of pro-inflammatory cytokines [120,121]. Peripheral leukocytes can infiltrate from the blood to the brain through ICAM-1 (Adhesion Molecule 1) and VCAM-1 (Vascular Adhesion Molecule 1) adhesion molecules, contributing to the epilepsy-related brain inflammation [122].

Altogether these findings indicate that restoration of BBB integrity can concur to dampen epileptogenesis. In principle, the ability of α-T in reducing neuroinflammation would promote BBB recovery, maintaining cerebral homeostasis and providing neuroprotection. In keeping with this hypothesis, the expression of claudin, a transmembrane protein involved in the formation of tight junctions, was stimulated by the treatment with α-T in KA epilepsy rats (250 mg/kg b.w. via i.p. for the first 4 days followed by 2 mg/kg for the remaining days up to fifteen from SE), suggesting effects of this vitamin also on BBB integrity restoration [29]. Accordingly, α-T treatment was proven to produce protective effects on BBB function following ischemic stroke occurrence [123] as well as vitamin E derivatives, such as α-Tocopheryl succinate, was demonstrated to inhibit the breakdown of BBB in experimental cerebral malaria [124].

3.3. Neurodegeneration and neurotoxicity induced by SE: the effect of vitamin E

Neuroinflammation and excitotoxicity can cause neuronal loss [40]. This type of lesion induces changes in hippocampal networks which accounts for epileptogenesis [40,125]. Thus, resizing the neurodegenerative processes by reducing inflammation and excitotoxicity would limit the aberrant alterations of hippocampal circuitry and the progression of epileptic disease with its detrimental effects on neurocognitive function. A wealth of in vitro and in vivo evidence [126–128] demonstrated the protective effect of α-T against neuronal death. Accordingly, α-T treatment (250 mg/kg b.w. via i.p. for 4 days) was effective in reducing neuronal degeneration and neurofilament degradation occurring after KA-induced SE [28]. Therefore, the findings in these recent studies strongly support the view that the effects of this vitamin in decreasing neuroinflammation and excitotoxicity are accompanied by a significant neuroprotective role, possibly contributing to reducing epileptogenic risk. In line with this, a strong reduction in excitability of hippocampal neuron networks and the resulting lack of spontaneous and drug-triggered epileptiform bursting events were clear-cut [29] and clinically relevant demonstrations of this neuroprotection role.

It is worth considering that neuronal death occurs only in some patients with epilepsy, as indicated by neuroimaging and pathological studies and by the evaluation of cell injury biomarkers as well [129,130]. Therefore, it is conceivable that besides overt neuronal death, more subtle mechanisms of seizure-induced brain injury can exist, which affect neuronal structure and function, to eventually induce the cognitive deficits often observed in patients with epilepsy. Dendritic spines are structures that are critical for synaptic transmission in the brain; these structures represent sites of contact receiving excitatory, glutamatergic synaptic inputs, and have been involved in mechanisms underlying synaptic plasticity and learning [131]. Thus, dendritic spine loss may constitute a mechanism of cognitive dysfunction. Guo et al. [132] demonstrated that KA-induced SE causes a remarkable loss of dendritic spines immediately after termination of seizures. This defect only in part reverses over 6 weeks of observation. In this epilepsy model, we also detected a conspicuous reduction in the number of dendritic spines of apical dendrites of hippocampal CA1 neurons four days after SE; α-T treatment (carried out as described earlier in this section) prevented this loss of dendritic spines the number of which was similar to non-epileptic control animals [28]. It is important to underline that the treatment with α-T began three hours after the pharmacological stimulation of SE, i.e., a time when the most of spines had already lost. These intriguing findings may lead to hypothesize that α-T influences the formation of new spines during the recovery phase after SE, although a combination with a prevention effect on spine loss cannot be ruled out.

α-T treatment also restored synaptophysin immunoreactivity in hippocampal CA1 neurons; this synaptic vesicle protein is involved in neurotransmitter release [133] and is also a marker of synapse loss in neurodegenerative pathologies [134] and defective synaptic contacts following a variety of experimental manipulations [135,136]. In this view, it is relevant to consider that a role of α-T in promoting synaptogenesis was previously highlighted in dentate gyrus of supplemented adult rats [67]; also in these experiments the effect of α-T treatment on synaptic dendritic profiles was associated with an enhanced immunoreactivity to synaptophysin. Together these observations may shed light on the importance of synapse damage in the neurobiology of epilepsies, prompting the need for investigation of therapeutic strategies that may help to prevent their negative effects. Vitamin E therapy might hold potential to improving synaptogenesis and the outcome of SE on cognitive functions, with effects that can be timely enough to represent a synapto-protective agent suitable for therapy of SE and other acute disorders of the brain in which preserving neuronal networks along with cell bodies is the actual, and so far elusive/frustrating, challenge. To our knowledge this represents a unique example of “synapto-protectant” in the area of micronutrients and physiological lipids of brain; other phenolics, such as resveratrol, have failed to demonstrate synapto-protective effects [137], while NAD + and some pharmacological agents, such as the Rho-kinase inhibitor Y27632, have provided positive effects, possibly acting on mechanisms alternative to antioxidant role of vitamin E.

The role of vitamin E as a stimulator of spine formation is completely new and open to mechanistic interpretation. Worth of investigation could be the role of this vitamin as phospholipase inhibitor [128] that may eventually impact on the phospholipid (PL) metabolism of the neuronal membrane during spine formation. Other hypotheses include the protection of unsaturated lipids on phospholipid structures (further discussed later) that may preserve membrane integrity thus preventing degenerative (free radical-mediated) events and the activation of lipid turnover and eventually membrane demolition processes at spine site by the activity of specific phospholipase isoenzymes [138].

3.4. Vitamin E and SE-induced aberrant neurogenesis

If on the one hand, SE induces neuronal death and synapse loss, on the other hand, it triggers the formation of new neurons. This latter process mainly occurs in hippocampal dentate gyrus where it generates ectopic granule cells that contribute to forming aberrant epileptogenic networks [128]. Preliminary results from our recent studies (unpublished data) revealed a significant reduction in the number of ectopic newborn neurons 30 days after KA-induced SE by the treatment with α-T (250 mg/kg b.w. via i.p. for the first 4 days followed by 2 mg/kg for the remaining days up to 30 days from SE); this finding was accompanied by an increase of new neurons properly located in the granule cell layer (Fig. 1). If confirmed these results would support a role for α-T therapy in counteracting aberrant neurogenesis.

Involvement of this vitamin in neurogenesis regulation in non-epileptic conditions has previously been demonstrated by our group investigating α-T supplemented rats and vitamin E-deficient rats, consistent with a role of α-T as a factor capable of promoting adult-generated granule cell survival and differentiation (for a review see [139]).

In keeping with this evidence, we recently observed that α-T treatment down-regulated the expression of miR-34a in the hippocampus of epileptic rats 30 days after KA-induced SE (unpublished data). This microRNA is crucial for neurogenesis process progression, being involved in morpho-functional maturation of adult-generated newborn cells in dentate gyrus and apoptosis as well [140,141]; moreover and consistently, experiments with miR-34a antagomir demonstrated that targeting miR-34a in epilepsy may contribute to increased neuronal survival and reduced neuronal death or apoptosis [142]. Importantly, the down-regulation of miR-34a was induced by α-T also in non-epileptic rats (unpublished data), indicating a direct influence of this molecule on microRNA expression already proposed in [143]. In addition and consistently, we also observed the influence of α-

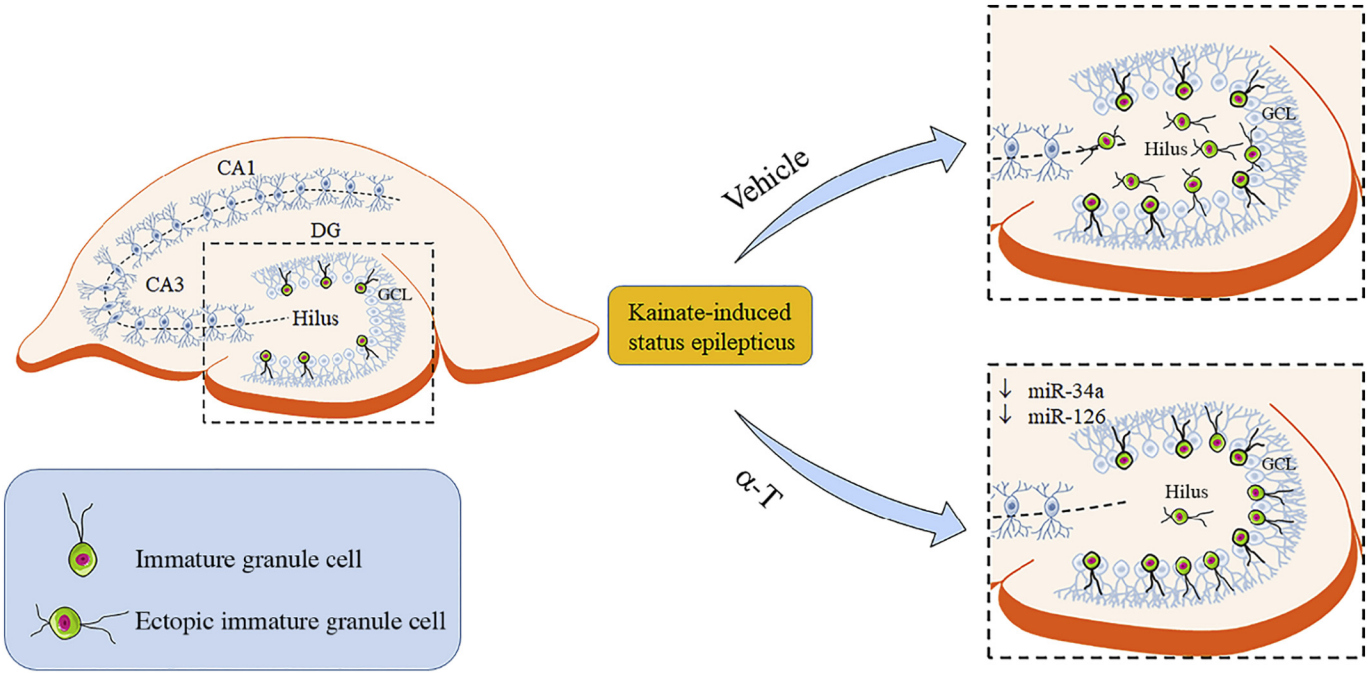

Fig. 1. Proposed effects of α-T treatment on aberrant neurogenesis promoted by the status epilepticus (kainic acid model of SE).
Ectopic immature neurons decrease in the hilus of dentate gyrus under α-T treatment conditions, while immature neurons properly localized in the granule cell layer increase. The microRNAs (miR-34a and miR-126) are down-expressed in epileptic rats treated by α-T, possibly promoting neuron differentiation and survival. DG: dentate gyrus; GCL: granule cell layer. This figure was prepared using the Neuroscience-PPT-Toolkit-Suite of Motifolio Inc., USA, and Servier Medical Art (https://smart.servier.com/).

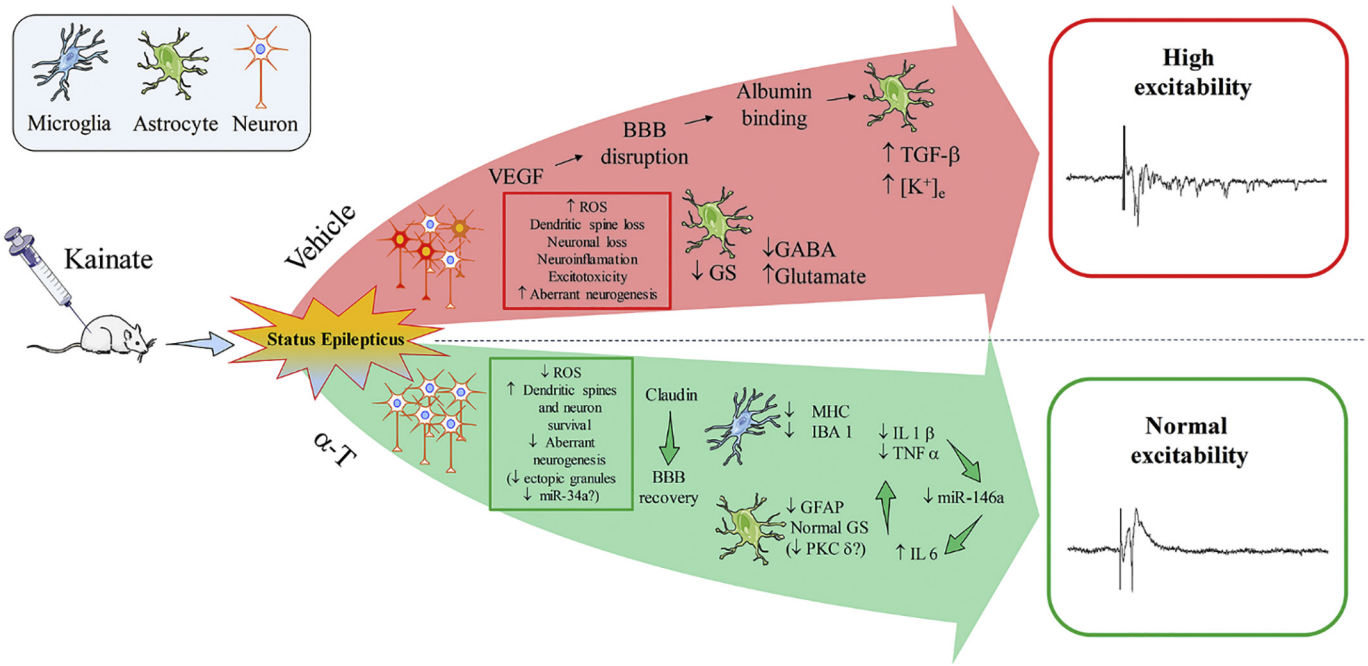

Fig. 2. Effects of α-T on underlying mechanisms of epileptogenesis and chronicization of epilepsy disease (exemplified from the KA model of SE). The main pathogenic factors of epileptogenesis investigated in KA induced SE are affected by α-T treatment during the latent period leading to recover normal neuronal network excitability. Details of α-T actions are described throughout the text. This figure was prepared using the Neuroscience-PPT-Toolkit-Suite of Motifolio Inc., USA, and Servier Medical Art (https://smart.servier.com/).
Abbreviations: VEGF: vascular endothelial growth factor; BBB: blood–brain barrier; TGF-β: Transforming growth factor β; $[K^+]_e$: extracellular potassium concentration; ROS: reactive oxygen species; GS: glutamine synthase; GABA: gamma-aminobutyric acid; MHC: major histocompatibility complex; IBA 1: ionized calcium-binding adaptor protein-1; IL 1β: interleukin 1 β; TNFα: tumor necrosis factor α; IL 6: interleukin 6: GFAP: glial fibrillary acidic protein; PKC δ: protein kinase δ; miR: microRNA.

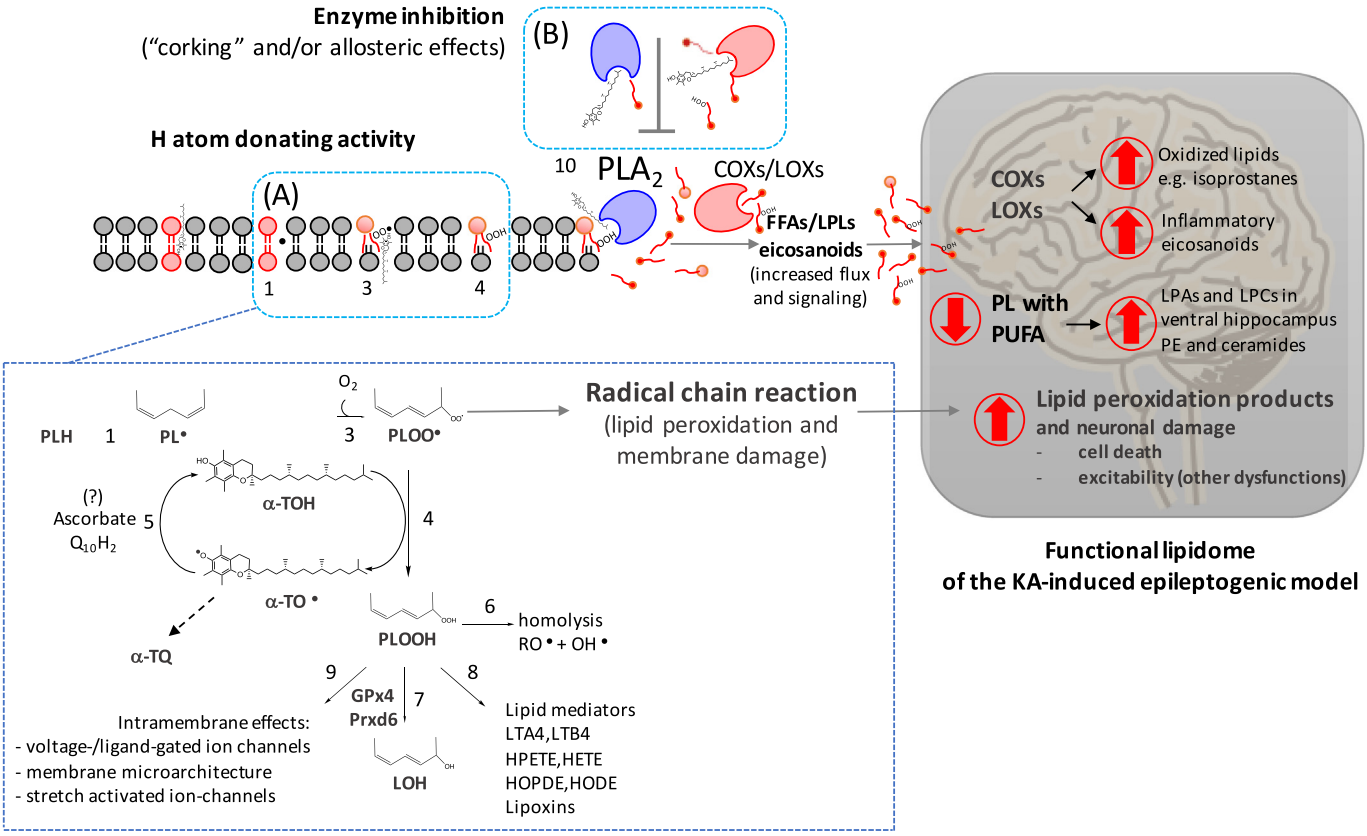

Fig. 3. A lipidomics view on the proposed (dual) neuroprotection mechanism of vitamin E (α-tocopherol) in the brain with epileptic condition (KA-induced SE model). Lipid peroxyl radicals and PLA2 activity are identified as main targets of the neuronal and glial cell membrane during SE. α-TOH can influence the brain lipidome during the (lipid-mediated) neurodegenerative process (insert A) by lowering the flux of PLOOH favouring the activity of membrane peroxidase enzymes. This may result in a lower PLA2 activation. Vitamin E may also directly interfere with PLA2 and COX/LOX activity by "corking" and allosteric mechanisms that are further described in the text. These effects of vitamin E synergize to attenuate the production of a series of lipid mediators with pathogenic roles in epileptogenesis and neurodegeneration, these include FFA and LPL, and then inflammatory/degenerative eicosanoids. The formation of a lipid radical (L$^{\bullet}$) and its reaction with molecular oxygen to form a lipid peroxyl radical (LOO$^{\bullet}$), are initiating steps in the peroxidation chain reaction membrane phospholipids (PLs). In this steps, an H radical abstraction process occurs on a polyunsaturated lipid residue (PLH) esterified to PLs (reaction 1) to form a PL$^{\bullet}$ or PLOO$^{\bullet}$ product (reaction 2). If not scavenged, these lipid-centered radicals propagate the radical chain. α-TOH is considered to represent the main H atom donating molecule of cellular membranes (reaction 3) and its oxidation generates the radical species α-TO$^{\bullet}$, a more stable radical compared with LOO$^{\bullet}$. α-TO$^{\bullet}$ regeneration to α-TOH may occur through other H atom transfer reactions that could be mediated by ascorbate or eventually by ubiquinol ($Q_{10}H_2$) (reaction 4). An increased oxidation rate of vitamin E or a poor re-generation of α-TO$^{\bullet}$ may lead to forming increased levels of the stable oxidation product α-tocopheryl quinone (α-TQ), a useful biomarker of lipid peroxidation and lipotoxicity in oxidant stress related human diseases. The lipid hydroperoxide (PLOOH) is a "reactive oxygen species" (ROS), since its hemolytic cleavage leads to two most reactive radicals, i.e., $^{\bullet}$OH and RO$^{\bullet}$ (reaction 5), thereby causing branching of the radical chain if they are not eliminated, e.g., by glutathione peroxidase (GPx4) or certain peroxiredoxins (Prxds), essentially Prxd6, forming a stable lipid alcohol (LOH) (reaction 6). LOOHs are not only dangerous chain-branching ROS, but they have also signaling activity or are themselves a source for other potent lipid mediators (reaction 7). Because of their hydrophilic –OOH group they cause a rearrangement of lipids in the membrane (reaction 8) which might affect membrane-associated enzymes, as can be deduced from the preferential cleavage of oxidized fatty acids by PLA2 and subsequent increased activity of oxidases (reaction 9).
(gray box on the right) These reactions influence the brain lipidome. An increased PLA2 activity stimulates an abnormal efflux of FFAs which are substrates of inflammatory enzymes, such as COXs and LOXs, resulting in increased generation of both oxidized lipids and inflammatory eicosanoids. PLA2 activity also influences the levels of PL that contain PUFAs on the neuronal cell membrane, and those of lysophospholipids (LPLs), with consequent changes in the phospholipidome of the brain with epileptic condition.
Abbreviations: LPLs: Lisophospholipids; FFAs: Free Fatty Acids; PL: Phospholipid; LPA: Lysophosphatidic acid; LPC: Lysophosphatidylcholine; PE: Phosphatidylethanolamine; PLA2: Phospholipase A2; COXs: Cyclooxygenases 2; LOXs: Lipoxygenases.

T on the hippocampal expression of miR-126 [29], which is a putative SRY (sex determining region Y)-box 2 (SOX2) regulator [144]. A decline of miR-126 has been observed independently from epileptic status [29], suggesting that α-T regulates miR-126 expression in brain increasing SOX2 expression. SOX2 is an important factor in maintaining self-renewal and pluripotency of stem cells and neural progenitors [145]. This miR is also involved in epidermal growth factor-like domain 7 (EGFL7) pathway regulation that controls the differentiation potential of neural stem cells [146].

4. Vitamin E and the redox disturbances (oxidant stress) of epilepsy

Excitotoxicity and neuroinflammation during a brain insult, such as seizure, are associated with increased production of reactive oxygen/nitrogen species (ROS/RNS) leading to an increased risk of oxidant stress [147]. Both free radical-generating oxidoreductases of inflammatory cells and the derangement of subcellular compartments of damaged neurons, and especially mitochondria, play a role in this condition ([148] and references therein). NADPH oxidase is the major source of ROS in injured brain microenvironment [149] and accumulating evidence draws attention to the importance of NOX2 and NOX4

isoforms in brain diseases, including epilepsy [49]. During a seizure, NOX2 activity is triggered in neurons by NMDA receptor activation, which exhibits a key role in epilepsy allowing intracellular $Ca2^{+}$ accumulation. Thus, rises of cellular ROS and Ca^{2+} act as potent triggers of mitochondrial permeability alteration, leading to mitochondrial swelling and then to cytochrome c release thus triggering the intrinsic pathway of apoptotic cell death ([49] and reference therein). NOX is also expressed by glial cells, mainly in astrocytes in which NOX2 and NOX4 are reported to play a major role [149].

In damaged mitochondria, electron leakage augments the formation rate of superoxide and consequently of H_2O_2 by the activity of mitochondrial and cytosolic superoxide dismutase, that are the Mn- and Cu/Zn-dependent isoforms of this enzyme, respectively. At the same time, superoxide can react with the radical NO produced by the cellular NO synthase, to form peroxynitrite, a relatively stable pro-oxidant molecule with proven role in neuronal cell damage [150,151]. Under conditions of persistent/severe cell damage, this flux of oxygen and nitrogen derived metabolites can augment to the point that it overwhelms the mitochondrial and extra-mitochondrial defence mechanisms responsible of maintaining the redox homeostasis of the cell thus leading to severe oxidant stress and further mitochondrial dysfunction and ROS/RNS production. Other consequences in this cascade of cellular events include an impaired calcium homeostasis, lipid peroxidation and membrane permeability alterations, and protein and DNA damage. All these aspects ultimately affect gene expression predisposing to cell cycle alterations, and eventually to senescence and/or cell death pathway activation [152].

Altogether, these maladaptive responses provide the mechanistic and toxicological signature of oxidanat stress in the neurodegeneration model of epilepsy and epileptogenesis.

4.1. Vitamin E: an effective fighter in counteracting oxidative stress and its outcomes in SE

In this scenario, a wealth of studies reported findings underpinning the potential benefits of natural antioxidants in preventing the neurodegenerative effects of oxidant stress [17,153]. These include vitamin E that was demonstrated to attenuate convulsive behavior in different pharmacological models of SE, such as pentylenetetrazol-, methylmalonate- and pilocarpine-induced SE when administered before seizure induction [23,25]. In some of these studies, the anticonvulsant action of vitamin E was accompanied by a lowering of brain lipid peroxidation and nitrite content, which may result in increased cytoprotection and neuronal survival as ultimate prevention mechanisms against hippocampal damage [23]. Other recent studies on pilocarpine-induced epilepsy model have confirmed the protection against oxidant stress of the brain, but not the anticonvulsant activity [154], pointing to different levels of outcome for different dosages of the vitamin and epileptogenic models.

In line with an efficient brain antioxidant effect of vitamin E, KA-induced epilepsy also produced a marked increase of oxidant stress indicators that was efficiently counteracted by the vitamin E treatment protocols described before in Section 3.1 and in [28,29,65]. More in detail, the increased lipid peroxidation assessed as thiobarbituric acid reactive substances (TBARS), observed four days after SE induction, was significantly prevented when α-T was administered either before [65] or immediately after SE induction [28]. Likewise, in another study [29] protein carbonyls increased in hippocampi of epileptic rats; this is a robust oxidant stress hallmark associated with protein damage and dysfunction by the reactive intermediates formed during molecular oxygen activation and the lipid peroxidation process [155,156]. Again, the treatment with α-T that lasted fifteen days post-ictal, significantly decreased the levels of carbonylation in hippocampal proteins [29].

Overall, these findings and other pieces of evidence in the literature [147,157] clearly indicate that oxidant stress initiated by SE is a relevant pathogenic event in the degenerative and dysfunctional processes of the brain with epilepsy condition.

Our studies on the KA model of SE demonstrated that lipid peroxidation persists long after the earliest post-ictal phases thus prompting the need for efficient secondary chemoprevention strategies to limit its damaging potential. α-T and its direct and indirect antioxidant effect(s) appear to be particularly useful to protect (before seizure) and then to prevent brain damages after the triggering event. Its prolonged efficacy and complete absence of side effects demonstrate great potential for a translation in the clinical management of oxidant stress-related complications of SE.

In this context, changes occurring in the brain lipidome during epileptic seizures, neuroinflammation and neurodegeneration appear to confirm the detrimental effects of lipid peroxidation. Available targeted and untargeted lipidomics procedures have recently provided insights into such changes as well as on functional roles of some lipid mediators in brain sub-regions during the acute phase of epileptic seizures. Altered phospholipid [158] and sphingolipid metabolism [159], increased peroxidation and cholesterol synthesis [160,161] have all been described as changes of the brain lipidome in the KA model as well as in other models of experimental epilepsy. Interestingly, epileptic seizures in the rat model of KA-induced epilepsy have been demonstrated to cause hippocampal reductions in most abundant phospholipid species containing polyunsaturated fatty acyl chains, with particular regard to acylated forms of phosphatidylethanolamines and ceramides [162], compatible with calcium-influx derived increase of phospholipase A (PLA) activity. The possibility that PLA activation would lead to abnormal PL degradation and subsequent formation of second messengers is a critical aspect in brain pathophysiology (discussed later in this section).

4.2. Mechanistic aspects of vitamin E "anti-peroxidation" function

The antioxidant and cytoprotection properties behind the positive experimental outcomes of vitamin E therapy in SE are based on mechanisms that are still far for being conclusively deciphered. Homeostatic and curative properties of this vitamin intended as a fat-soluble antioxidant factor, appear to depend on its functional interplay with other detoxification and lipid metabolism systems strategically distributed in the different cellular lipid compartments. These include some phospholipase isoforms, the oxidases of the LOX and COX families of isoenzymes, phospholipid hydroperoxide peroxidases - with the glutathione peroxidase isoenzyme GPx4 as the most prominent player - and lipid catabolism enzymes that include several members of the cytochrome P-450 superfamily. The integrated function of these systems controls the flux of polyunsaturated fatty acid (PUFA) substrates throughout enzymatic and non-enzymatic processes that generate several classes of eicosanoids, isoprostanes, and neuroprostanes with multiple effects on neural, immuno-inflammatory and vascular functions [163]. These compounds are generated by a variety of lipid metabolizing pathways that act as direct effectors of signal transduction in the nervous system through interactions with membrane proteins that include ion channels, cytoskeletal proteins, and G-protein-coupled receptors, as well as with nuclear receptors (reviewed in [164] and further discussed in [165]). Peroxide formation and PLA2 activity may potentially affect the membrane-associated signaling function with other mechanisms that include changes in the architecture and biophysical properties of phospholipid membranes. In fact, the molecular geometry of phospholipid peroxides and that of lysophospholipids (LPLs) and free fatty acids (FFAs) formed during their PLA2-mediated hydrolysis, is quite different from the parent phospholipid molecules present on the membrane before the peroxidation insult. Furthermore, other lipid-based neuromodulators generated by PLA2 activation during membrane lipid peroxidation include FFAs themselves - e.g. arachidonic acid (AA) and docosahexaenoic acid (DHA) and LPLs (e.g. lysolecithin) [164]. Now the progress of lipidomic technology (comprehensive description of methods and applications of this technology

to lipid oxidation studies can be found in [166]) is expected to expand the identification of these bioactive lipids together with other lipid metabolism products with important function in the pathophysiology of human brain.

Non-enzymatic antioxidants integrate the phospholipid metabolism/homeostasis system of the brain, but their actual role is still poorly understood. α-T appears to be (by far) the most important antioxidant of PUFAs in the plasmalemma and a contribution to lipid homeostais of other cellular membranes has also been suggested in some studies [167]. The possibility that in the mitochondrial membrane the coenzyme Q may surrogate or even overcome the importance of α-T as (direct or indirect) H atom donor during chain-breaking reactions cannot be ruled out. The relationship between these two redox-active molecules is now under investigation for important implications in mitochondrial lipid signaling and regulation of cell death programs, especially ferroptosis [168,169].

Vitamin E, under the form of α-T, reaches the brain tissue to biologically significant levels, i.e. the levels that appear to provide enough H atoms to influence to some extent the peroxyl radical flux and the integrity of the tissue. Also, the redox recycling and H atom donating ability of this vitamin (one of the highest among natural phenolics [167] and food lipid antioxidants, such as the synthetic compound butylated hydroxytoluene that has a 200-fold slower capability to scavenge peroxyl radicals compared with α-T [170]), should be very efficient in the brain. In this organ, antioxidants have to cope with a sustained oxygen metabolism and relatively poor antioxidant protection of PUFAs that are present in brain tissue at one of the highest concentrations of the entire organism, especially the ω-3 species DHA [164,171]. In other words, the proposed role of α-T in preventing lipid peroxidation and oxidative stress of the brain should be associated with a brain-specific metabolism and function of this vitamin. Accordingly, vitamin E half-life in this tissue is slower than in others [172,173], consistent with the evidence that vitamin E is actively retained and protected from autooxidation in the brain. The tissue-specific mechanism behind this evidence is likely mediated by the α-tocopherol transfer protein (α-TTP), which has been shown to bind and traffic α-T within the brain [174]. Cerebellar α-TTP expression is regulated both by oxidative stress and vitamin E status [175,176]. For example, α-TTP is markedly elevated in brain samples of patients affected by neurodegenerative diseases, such as Alzheimer's disease (AD) [177], compatible with a modified brain metabolism of the vitamin or an increased demand of antioxidant protection. This degenerative condition is confirmed to occur in animal models of premature aging in which vitamin E supplementation mitigates brain oxidant stress and neuronal cell loss [178]. Again, a chronic deficiency of vitamin E can impair cognitive functions via dysregulation of brain lipid composition and energy metabolism that occur early in the developmental brain [179]. Decreased concentrations and/or increased peroxidation of PUFA, especially DHA, could be responsible for these effects leading to altered brain phospholipid and lysophospholipid composition, and perturbed energy (glucose/ketone), phosphatidylcholine and choline/methyl-donor metabolism [179].

Now, despite this important role of α-T in brain lipid homeostasis, the actual mechanism of action of this vitamin in this organ remains elusive in most of its aspects [174]. As introduced earlier in this section, its H atom donating activity and the relative abundance compared with that of membrane PUFAs, support a role for α-T as main chain breaker of the plasmalemma [180]. The peculiar structure of the molecule appears to allow a strategic distribution on the bilayer with its phenolic moiety (i.e. the chromanol ring) oriented to intercept the chain of lipid peroxyl radicals ($LOO^{\bullet}$) for the H atom donation reaction [181,182]. In fact, the alkoxyl radical group confers increased polarity to the lipid chain that folds toward the polar (external) edge of phospholipid layer to physically interact with the hydroxyl group in position 6 of the chroman ring. This type of distribution has been proposed to strategically favor the activity of the vitamin at the site of $LOO^{\bullet}$ formation also allowing the tocopheryl radical to undergo redox restoration by cellular and extracellular co-antioxidants. Among the latter, kinetics and experimental (H atom transfer) data strongly suggest that ascorbic acid is the main water soluble small molecule antioxidant that efficiently interacts on one side with α-T to provide efficient transmembrane electron transfer and protection from lipid peroxidation, and on the other with NADPH-dependent (GSH or Trx mediated) systems that recycle ascorbate from its oxidized form [183–185]. Also, the formation of membrane microdomains rich in PUFA may favor a strategic localization and antioxidant activity of α-T [182]. These microdomains also tend to increase the bilayer's curvature, a physical process that may lead to specific interactions with LOOH processing enzymes and especially PLA2 (discussed earlier in this subsection and in [165]), so that the activity of these proteins may occur in a concerted way. Here we suggest that vitamin E represents a conditional co-factor for these functional complexes of the cellular membrane, such conditional role could depict better than others its essentiality as a micronutrient vitamin.

In keeping with this view, vitamin E similarly to other radical scavengers/trappers influences the flux of LOOH that derives from both the spontaneous and enzymatic (oxygenase-mediated) formation of $LOO^{\bullet}$ on the cellular membrane [186]. Effects of this vitamin on the peroxidation activity of all main LOX isoenzymes have consistently been demonstrated and appear to involve both the radical scavenging mechanism (i.e., the H atom donor activity) and a physical interaction with the enzyme essentially competing with the polyunsaturated lipid substrate for the binding in the catalytic pocket, also identified as to “corking” mechanism [187]. In this context, however, very recent and compelling evidence is suggesting that autoxidation mechanisms might play a major role in lipid hydroperoxide formation and signaling throughout cell pathways [186], consistent with an apical position of vitamin E redox in the pecking order, or hierarchy, of factors that control this important aspect of PUFA' metabolism. To further support this “redox-tionistic” view, Hinman et al. in a recent study demonstrated that besides α-T, also the main product of its reaction with $LOO^{\bullet}$, α-tocopheryl quinone (α-TQ), can inhibit the 15-LOX mediated lipid signaling through the ferroptotic death program of striatal cells. This effect was found to be more potent compared with that of α-T and it occurred when α-TQ was recycled to its hydroquinone form (α-TQH_2), a process that physiologically can occur in the cell by the activity of (NADPH-dependent) quinone oxidoreductases [188].

Worth of note, the same dual mechanism of action (antioxidant and non-antioxidant) described for LOX isoenzymes appear to apply for the modulation effect that vitamin E compounds exert on PLA2 activity, the enzymatic step that hydrolyzes membrane phospholipids to provide PUFA substrates to LOXs and COXs ([165,189]). In fact, a “corking mechanism” was also described for the PLA2 inhibitory activity of α-T (even supported by crystallographic data [190]). On the other hand, peroxyl radical scavengers are capable of lowering the release of arachidonic acid of inflammatory cells and neurons, lowering PLA2 activation possibly with an indirect mechanism [191], likely the same mechanism that brings this molecule to transversally influence all LOX isoforms ([192] (further discussed earlier and in [186]). Accordingly, Grau and Ortiz suggested that α-tocopherol, whose chromanol group resides higher in the membrane (toward the surface) and inhibits PLA2 the most, has an effect on the substrate - i.e., the membrane - and not on the enzyme itself [193]. Worth of note, in these studies the grade of PLA2 inhibition by α-T and other tocopherols (the β-, γ- and δ-tocopherol isomers) correlated well with their biological activity and location in the bilayer. At the same time, cholesterol did not inhibit PLA2 activity at concentrations even higher than those of these tocopherols, suggesting that the effect of tocopherols was not due to membrane fluidity changes.

Lerner et al., described a hippocampal-specific increase of cytosolic PLA2 (cPLA2) activity in neuronal cells that only occurred in the ventral hippocampus in which phosphatidylcholine (PC) species decreased.

This, in turn, was suggested to enhance lysophosphatidic acids (LPAs) and lysophosphatidylcholine (LPCs) that are important lipid signaling molecules; on the contrary, an increase of phospholipid levels was observed in the dorsal hippocampus despite the increased cPLA2 activity. These findings appear to suggest different distribution and activity of this PLA isoenzyme in the various brain regions, with effects on PL metabolism and functions that may also involve other pathways and enzymatic players [194]. Likely, the increased cPLA2 in dorsal hippocampus increases the release of arachidonic acid from the position 2 of glycerol molecule. This is a substrate for cellular oxygenase isoenzymes, and especially COX2, to produce inflammatory eicosanoids that are typically over-expressed in the hippocampus during epilepsy-associated neuroinflammation [194].

4.3. Vitamin E bioactivation (pro-vitamin) mechanism of action

As a general observation, tocotrienols appear to be more potent inhibitors of PLA2/LOX pathways compared with tocopherols [187,189], consistent with different cellular bioavailability/distribution and protein-interaction properties of saturate and unsaturated forms of brain vitamin E [195]. Beside to neuroprotection discussed earlier in Section 4.2 and recently reviewed elsewhere [196], these differences have also described to influencing other effects of vitamin E compounds, such as anti-cancer and immunomodulation [197].

The reason behind these vitamer-specific effects on lipid metabolism and signaling remains unknown and is surely worth of investigation. Tocotrienols and tocopherols both act as H atom donors, but their relative abundance in tissues may suggest minor roles of the former compared to tocopherols, and especially to α-T. Also, specific molecular targets (for example, a receptor or a sensor) that may selectively amplify the activity of tocotrienols in tissues have not been identified so far. However, tocotrienols, similarly to dimethyl tocochromanols, are highly metabolized forms of vitamin E, which is a major reason why α-T is preferentially retained in tissues while these other forms are present to trace amounts. However, a series of recent studies are pointing to a direct role of vitamin E metabolites in its regulatory properties, thus confirming the original hypothesis by some of us about the correspondence of this metabolism with a bioactivation process to transform the vitamin precursor in a series of active and more potent metabolites [198]. These recent studies are now suggesting that similar to the other lipophilic vitamins, such as vitamin A and D, vitamin E is a pro-vitamin (recently reviewed elsewhere in [17,43,188]). For example, long-chain enzymatic metabolites of α-T are potent inhibitors of 5-LOX ([199] and references therein). Again, the main free radical-derived metabolite of vitamin E, namely α-TQ is reported to inhibit with a redox mechanism (i.e., by the formation of its redox couple α-TQ/TQH_2) the 15-LOX dependent lipid signaling of striatal cells exposed to oxidant stress and to prevent cell damage and death in cells isolated from patients affected by primary neurodegenerative mitochondriopathies, such as Friedreich's Ataxia and Leigh syndrome ([188] and references therein). Tocopheryl phosphate is another physiological metabolite of α-T [200] with signaling and gene regulation effects in different cell models, including neurons, and in vivo in the brain tissue [201–203]. Furthermore, work by some of us demonstrated that the CYP-450 derived metabolite α-13'OH is a potent inducer of peroxisome proliferator-activated receptor γ (PPAR-γ) expression in hepatocytes [204]. That activity was also recently confirmed in astrocytes in which the effect of this nuclear receptor with key role in brain lipid metabolism and neuroprotection, also influences ApoE expression and function (Torquato P., Borras C., Vina J., Galli F., unpublished data).

Both enzymatic and free radical-derived long-chain metabolites have been detected at low nanomolar concentrations in human blood (between approx. 5 and 50 nM depending on the intake of α-T) [205–208], and the possibility that they also form in the brain is more than obvious since the enzymatic and non-enzymatic players responsible for their formation are present in the brain tissue (reviewed in [17]).

If this view is confirmed, we should adopt a major rethinking of vitamin E function. It is conceivable that α-T is not a simple antioxidant protector of cell membrane integrity, i.e. a H atom donor and chain breaker of unwanted lipid peroxidation reactions. Rather this vitamin, and/or some of its metabolites, appear to influence the physiological flux of lipid peroxides influencing at the same time the expression and activity of a series of lipid metabolism genes. These are important to control lipid peroxidation and its effects on the structural integrity of the cell membranes and the formation of bioactive metabolites that sustain inflammatory, vascular and metabolic responses of tissues and organs, the brain is included. In other words, preventing an excess of damage and regulating the signaling function of PUFA are two faces of the same coin that is the physiological and concerted role of vitamin E and the PLA2/oxidoreductase system.

5. Conclusions

Over the last years, numerous antiepileptic drugs have been produced. Nevertheless, several unmet clinical needs remain, including the lack of treatments to prevent the development of epilepsy in patients at risk, possibly by reducing seizure-triggered maladaptive neural plasticity underlying epileptogenic processes. Evidence presented and discussed here underscores the relevant role of vitamin E in affecting the pathogenic "triad" of epilepsy, inducing to seizure-evoked cell death, increased susceptibility to neuronal synchronization and network alterations (Fig. 2). α-T represents the form of vitamin E with the highest efficacy supported by brain bioavailability and activity data (that are critically evaluated elsewhere [17,209]). However, a role for other vitamin E forms in pharmacological protocols of neuroprotection during SE cannot be excluded; this include, for instance, chemoprevention effects for α-tocotrienol described in models of glutamate-induced excitotoxicity and neurodegeneration.

In point of fact, excitotoxicity, neuroinflammation, and oxidative stress are all affected in pre-clinical models by the supplementation with α-T, thus representing an efficient and safe procedure to counteract maladaptive plasticity promoted by these pathogenic factors. Mechanisms implicated in α-T-mediated actions keep on to be under investigation; available evidence on the KA-induced model of epilepsy examined in this review paper, suggests the participation of different and possibly complementary tocopherol's properties. Besides the well-known antioxidant (lipid peroxyl radical scavenging) function, its ability to directly influence the lipidome and then the lipid signaling and gene response of neuronal and glial elements are now emerging as the actual neuroprotection function of this fat-soluble vitamin (Fig. 3). Mechanistically, a complex and so far poorly understood series of regulatory interactions with key players of membrane PL metabolism appear to explain this function. These include effects on PLA2, GPx4 and LOX isoform' activity. These are responsible for regulating the flux of oxygenated lipid mediators in both the healthy brain and brain with epilepsy condition.

New insights into the neuroprotection effects of vitamin E are expected to come from emerging evidence in literature of a bioactivation mechanism for this vitamin. This generates a series of enzymatic and free radical-derived long-chain metabolites with potent anti-inflammatory activity and effects of modulation on lipid metabolism and detoxification pathways [17,43]. If confirmed, these emerging aspects may lead to change our view on vitamin E essentiality and function in the brain and in other organs, indicating a pro-vitamin mechanism of action. This stimulating view originally proposed by some of us more than a decade ago [198], is now emerging as an hot-topic in functional lipidomics wich is expected to spur translation of vitamin E therapy in seizure-induced brain damage and epileptogenesis, as well as in other brain disorders.

As a practical information, bioactive metabolites form and maintain significant steady-state concentrations in blood and liver when supra-

nutritional dosages of vitamin E are administered for a sufficient time (> 1 week) to humans and animals [206,207,210], compatible with treatment conditions of previous clinical trials that demonstrated positive effects on patients affected by drug-resistant epilepsy treated for one month with a dosage of 600 IU/day [16] or for 6 months with 400 IU/day [18]. Hopefully, these new aspects of vitamin E metabolism and function may also help to explain conflicting results obtained in other clinical trials (discussed in Section 1).

In this respect, assessing vitamin E metabolism and its interaction with other frames of patient's lipidome (such as those associated with peroxidation and inflammatory lipids), is now representing a groundbreaking opportunity for neuroscientists and potent tool to design the next generation of clinical trials on vitamin E therapy. A relevant example of these analytical advancements is the possibility to reliably measure in human plasma and solid tissues α-TQ [211]. This lipid peroxyl radical-derived product of vitamin E oxidation is unique as surrogate indicator of lipid peroxidation linking with a causal association vitamin E' antioxidant function and oxidant stress-induced lipid damage that can be confirmed with other surrogate indicators, such as 4-hydroxynonenal [211]. These aspects are even more important if we consider that experimental data in animal models and clinical trials are increasingly demonstrating that epilepsy may represent a lipid metabolism disorder of the brain and possibly of other organs, such as the liver and adipose. In this scenario, vitamin E' regulatory activities offer a unique option to improve the clinical management of drug-resistant epilepsy, which unlikely remains an orphan disease.

In spite of consistent preclinical findings, the level of evidence in clinical trials is not sufficient to gather vitamin E into clinical guidelines as chemoprevention or add-on therapy, to reduce epileptogenesis, i.e. to help constrain brain lesions (neuroprotection) and the risk of spontaneous recurrent crisis in patients who underwent a first seizure episode. Besides neuroprotection, preclinical studies also suggest applications in seizure management, prevention of cognitive impairment and optimization of anti-epileptic drug therapy that are all important aspects worth investigating further in clinical trials and more accurate dose-response studies.

Transparency document

The Transparency document associated with this article can be found, in online version.

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Conflict of interest statement

The authors declare that they have no conflict of interests.

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