Free Radical Research, October 2011; 45(10): 1136–1142

informa
healthcare

Dietary supplementation with α-tocopherol reduces neuroinflammation and neuronal degeneration in the rat brain after kainic acid-induced status epilepticus

MICHELE BETTI[1], ANDREA MINELLI[1], PATRIZIA AMBROGINI[1], STEFANO CIUFFOLI[1], VALENTINA VIOLA[2], FRANCESCO GALLI[2], BARBARA CANONICO[1], DAVIDE LATTANZI[1], EVELIN COLOMBO[3], PIERO SESTILI[3] & RICCARDO CUPPINI[1]

[1]*Department of Earth, Life and Environmental Sciences, University of Urbino 'Carlo Bo', I-61029 Urbino, Italy,* [2]*Department of Internal Medicine, University of Perugia, I-06100 Perugia, Italy, and* [3]*Department of Biomolecular Sciences, University of Urbino 'Carlo Bo', I-61029 Urbino, Italy*

(Received 28 March 2011; Accepted 13 June 2011)

Abstract

Vitamin E (as α-tocopherol, α-T) is proposed to alleviate glia-mediated inflammation in neurological diseases, but such a role in epilepsy is still elusive. This study investigated the effect of α-T supplementation on glial activation, neuronal cell death and oxidative stress of rat brain exposed to kainate-induced seizures. Animals were fed for 2 weeks with a α-T-enriched diet (estimated intake of 750 mg/kg/day) before undergoing status epilepticus. Compliance to supplementation was demonstrated by the remarkable increase in brain α-T. Four days after seizure, brain α-T returned to baseline and lipid peroxidation markers decreased as compared to non-supplemented rats. Status epilepticus induced a lower up-regulation of astrocytic and microglial antigens (GFAP and MHC II, respectively) and production of pro-inflammatory cytokines (IL-1β and TNF-α) in supplemented than in non-supplemented animals. This anti-inflammatory effect was associated with a lower neuronal cell death. In conclusion, α-T dietary supplementation prevents oxidative stress, neuroglial over-activation and cell death occurring after kainate-induced seizures. This evidence paves the way to an anti-inflammatory and neuroprotective role of α-T interventions in epilepsy.

Keywords: *Vitamin E, bioavailability, epilepsy, oxidative stress, astrocytes, microglia, neuroprotection.*

Introduction

Sustained astrocytic hypertrophy and proliferation, as well as excessive or prolonged microglial activation, are thought to be involved in epileptogenesis [1]. A rapid induction of interleukin-1β (IL-1β), IL-6 and tumour necrosis factor-α (TNF-α) occurs in microglia and astrocytes after acute seizures, followed by a cascade of downstream inflammatory events [1–4]. In rodents, IL-1β and TNF-α alter neuronal excitability and increase the probability of seizure generation [5,6] and contribute to neuronal cell loss and astrogliosis [6,7]; consistently, microglial activation and elevated pro-inflammatory cytokine production in the immature brain may lead to long-term increase in seizure susceptibility [8,9].

Antioxidant compounds such as vitamin E (VitE) have been proposed in clinical and pre-clinical studies as an effective treatment in neurological diseases such as stroke and neurodegenerative pathologies, in which inflammatory events and oxidative stress have a clear causal role [10]. α-tocopherol (α-T), i.e. the form of VitE with highest *in vivo* bioavailability [11,12], has also been reported to prevent neurotoxicity and neurological symptoms in rat models of chemically-induced epilepsy. Convulsive behaviour was attenuated in pentylenetetrazol-, methylmalonate- and pilocarpine-induced seizures [13,14], while brain lipid peroxidation and nitrite content were lowered [13,14] with resulting lower hippocampal damage and increased survival [14]. Although these beneficial

Correspondence: Dr Michele Betti, Department of Earth, Life and Environmental Sciences, Section of Physiology, University of Urbino 'Carlo Bo', Via Ca' le Suore 2, 61029-Urbino, Italy. Tel: + 390722304286. Fax: + 390722304226. Email: michele.betti@uniurb.it

ISSN 1071-5762 print/ISSN 1029-2470 online © 2011 Informa UK, Ltd.
DOI: 10.3109/10715762.2011.597750

properties have been largely attributed to prevention of free radical-induced neurotoxicity, VitE has been shown to down-regulate astrocytic and microglial reactivity and glia-mediated inflammation both *in vitro* and *in vivo* [15–19], which suggest antioxidant independent mechanisms. Accordingly, VitE is known to influence signalling and transcriptional activity of neuronal and glial cells through antioxidant-independent events regulating main biological responses such as cell cycle progression and apoptosis, mitochondrial function and detoxification processes [20]. Based on this background, an *in vivo* role of VitE in modulating neuroglial activation in epilepsy can be foreseen.

To address this issue, glial activation occurring after kainate-induced status epilepticus (SE) was investigated in the rat brain after supplementation with high dietary doses of α-T. The expression of astrocytic and microglial antigens, i.e. glial fibrillary acidic protein (GFAP) and major histocompatibility complex II (MHC II), respectively, as well as pro-inflammatory cytokines (IL-1β and TNF-α) production were evaluated in the whole brain. Oxidative stress and neurodegeneration were also investigated. Here, VitE-mediated effects were assessed 4 days after seizures; this time point was chosen since it corresponds to the beginning of a transition phase (spanning between 3–7 days after SE) in which, although in absence of EEG and behavioural seizures, specific neuroinflammatory responses are still active as an underlying event in the onset of chronic epilepsy [2,21].

Materials and methods

Male Sprague-Dawley albino rats (Charles River, Italy; ~ 200 g) were used in these studies. Animal care and experimentations were done in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). Rats were either given standard diet (65 mg/kg pellet α-T; $n = 15$) or fed with a diet enriched in the natural RRR-α-T form (10 g/kg pellet; Laboratori Dottori Piccioni, Italy; $n = 15$). Seizures were induced in both supplemented (S-S; $n = 11$) and non-supplemented (NS-S; $n = 11$) rats by single i.p. injection of kainic acid (KA, 5 mg/kg); other animals were sham-injected with physiological saline (S-C and NS-C for supplemented and non-supplemented animals, respectively; $n = 4$ for both groups). α-T diet was administered from 14 days before seizure induction and only animals which developed a full status epilepticus (SE) [22] were used for the study. Four days after KA injection, rats were anaesthetized with sodium pentothal (45 mg/kg) and perfused transcardially with ice-cold physiological saline and the forebrain was quickly removed.

Twenty rats (S-S and NS-S, $n = 6$ each; S-C and NS-C, $n = 4$ each) were used for determining α-T accumulation and immunoblotting experiments (one hemisphere for each method). Forebrain tissue was immediately frozen in liquid N_2 and measurement was performed by HPLC-ECD analysis as previously described [23]. For immunoblotting, the following anti-sera were used: mouse monoclonal antibodies against major histocompatibility complex (MHC) class II (MHC II or OX-6; Abcam: Cambridge, UK), glial fibrillary acidic protein (GFAP; Sigma: Saint Louis, MO, USA) and interleukin-1β (IL-1β; Santa Cruz Biotechnology: Santa Cruz, CA, USA); rabbit polyclonal anti-tumour necrosis factor-α (TNF-α; Sigma) and anti-actin (Sigma). The forebrain was homogenized and samples were prepared as previously described [23]. Blots were incubated with specific primary anti-sera (1:1000) and subsequently with the appropriate secondary antibodies conjugated with horseradish peroxidase (1:3000; Bio-Rad, Milan, Italy). Immune complexes were visualized using an enhanced chemioluminescence Western blot analysis system (Amersham–Pharmacia, Milan, Italy). Blot images were then digitized (Chemidoc, Bio-Rad) and areas of all labelled bands were quantified using the computerized imaging system software (QuantityOne; Bio-Rad). After visualization of immunolabelled bands, nitrocellulose membranes were stripped and reprobed with anti-actin antibody (1:200). In each series, relative optical densities (arbitrary units) were normalized for densitometric values obtained from actin-labelled bands (taken as control).

For cytometry, a 1.5 mm-thick coronal slice was dissected from the mid-left hemisphere (NS-S and S-S, $n = 5$ each) and disrupted in a glass homogenizer [24]. Cells obtained from mechanical disgregation were counted: cell suspensions between $3.5–7 \times 10^6$ cell/ml were overlaid on the top of a gradient containing Ficoll Hypaque [25]. After centrifugation, the cell ring was harvested and washed in PBS to decrease debris fraction. The pellet was then divided into two parts: one was immediately processed for Carboxyfluorescein diacetate (CFDA)/Propidium Iodide (PI) staining, performed on fresh cells [26], whereas the latter was fixed in paraformaldehyde 2%, washed in PBS and stained with FluoroJade® (FJ) to label degenerating neurons [2]. The protocol for FJ staining was adapted for the first time for flow cytometry (FC): fixed cells were immersed in 70% alcohol for 2 min, rinsed in distilled water and then incubated in 0.06% potassium permanganate for 10 min. After rinsing in distilled water for 2 min, cells were incubated in 0.0004% FluoroJade® solution in 0.1% acetic acid for 20 min, washed in distilled water and analysed by FC.

For oxidative stress evaluation, a 500 mg-weight coronal slice was separated from the mid of the right hemisphere of the same rats used for cytometry analysis. Levels of MDA were measured with the thiobarbituric acid reactive substances (TBARS) spectrophotometric assay using 1,1',3,3'-tetraethoxypropane

as a standard, as previously described [27]. Briefly, tissue samples were homogenized in 0.5 ml of a solution containing 1.15% w/v KCl. The reaction mixture contained a 0.1 ml aliquot of sample homogenate, 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid solution and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid. This mixture was diluted to 4 ml with distilled water and heated at 95°C for 60 min. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol were added and the mixture was shaken vigorously. After centrifugation, the absorbance of the organic layer was measured at 532 nm.

Statistical analyses were performed by one-way ANOVA followed by LSD Post-hoc tests for multiple comparisons or unpaired Student's *t*-test, with $p = 0.05$ as a significance threshold.

Results

Only rats which underwent a full status epilepticus were considered for the present study. In these rats, no significant differences between groups were found in the latency to the first wet-dog shake (NS-S 29.6 ± 1.72 min; S-S 28.4 ± 4.11 min; Student's *t*-test, $p = 0.79$) and to the onset of tonic-clonic activity (NS-S 69.2 ± 4.49 min; S-S 77.2 ± 4.1 min; Student's *t*-test, $p = 0.22$). The duration of SE was also similar, with seizures carrying on for ~ 4 h in both supplemented and non-supplemented rats.

Based on the average amount of pelleted food consumed by supplemented rats (15 g/day), the estimated daily intake of α-T was 150 mg (750 mg/kg/day). Animals fed for 2 weeks with this α-T diet exhibited a 3-fold increase in brain levels of α-T (Figure 1), thus revealing a remarkable brain bioavailability of orally supplemented VitE, in line with previous reports using longer-term dietary supplementation protocols [28]. Four days after SE induction, brain α-T concentration in supplemented rats decreased, returning to the same levels observed in non-supplemented animals after seizures (Figure 1). In both groups, brain α-T level found after seizures was significantly lower than in normally-fed rats not exposed to SE (Figure 1).

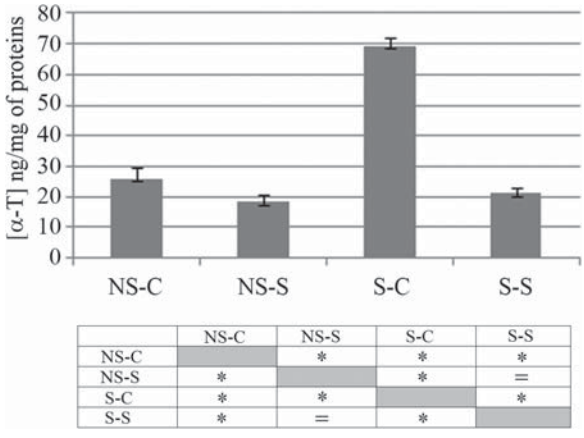

Figure 1. Effects of VitE supplementation and KA-induced status epilepticus on α-tocopherol brain concentration. Histogram reports α-T concentration levels measured in the whole brain by HPLC-ECD and expressed in ng/mg of proteins (means ± SD) in rats exposed to different experimental conditions: *NS-C*, non-supplemented sham-injected; *S-C*, supplemented sham-injected; *NS-S*, non-supplemented SE-exposed; *S-S*, supplemented SE-exposed. Statistical analyses performed by one-way Anova and LSD post-hoc test are tabulated below: * $p < 0.05$.

Four days following seizures induction, the oxidative status was measured with the TBARS assay in S-S and NS-S rats. A significant amount of lipid peroxides could be detected in both groups (Figure 2); however, S-S rats were characterized by a lower level of TBARS (Figure 2), reflecting a reduction in lipid peroxidation in supplemented animals.

Densitometric analyses of immunoblots revealed that, 4 days after SE, in NS-S animals the expression levels of GFAP and MHC II, markers of reactive astrogliosis and microglial activation, respectively, were both markedly increased (from 2- to 3-fold). The expression of pro-inflammatory cytokines IL-1β and TNF-α was also up-regulated (Figure 3).

In S-S rats, all the neuroinflammatory markers were expressed to a significantly lower extent than in NS-S (Figure 3).

To verify whether α-T treatment *per se* could regulate the expression of neuroinflammatory markers also in the absence of seizures, the same analyses were performed in S-C. Results showed that GFAP and IL-1β expression levels were unchanged, while MHC II and TNF-α were significantly reduced following α-T supplementation (Figure 3).

Cytometric analyses were used to evaluate cell death. CFDA/PI staining of cells from NS-S samples highlighted higher percentages of double positive cells CFDA/PI than S-S (Figure 4A). Quadrant markers were established taking into account differences in cellular self-fluorescence on FL1 and FL3 (data not shown). In NS-S samples, viable cells were in a lesser percentage than in S-S (NS-S $63.3 \pm 7.74\%$; S-S $88.7 \pm 1.25\%$; Student's *t*-test: $p = 0.012$), suggesting that α-T supplementation can protect brain cells. FJ

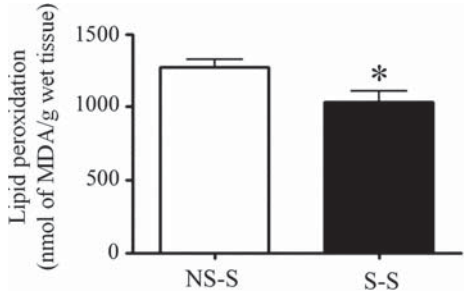

Figure 2. Effect of α-tocopherol supplementation on the levels of lipid peroxidation in brain exposed to KA-induced status epilepticus. Lipid peroxidation was determined 4 days following seizure attack with the TBARS assay. NS-S, non-supplemented SE-exposed; S-S, supplemented SE-exposed. Data represent the mean ± SEM of four separate determinations. Student's *t*-test: $p < 0.05$.

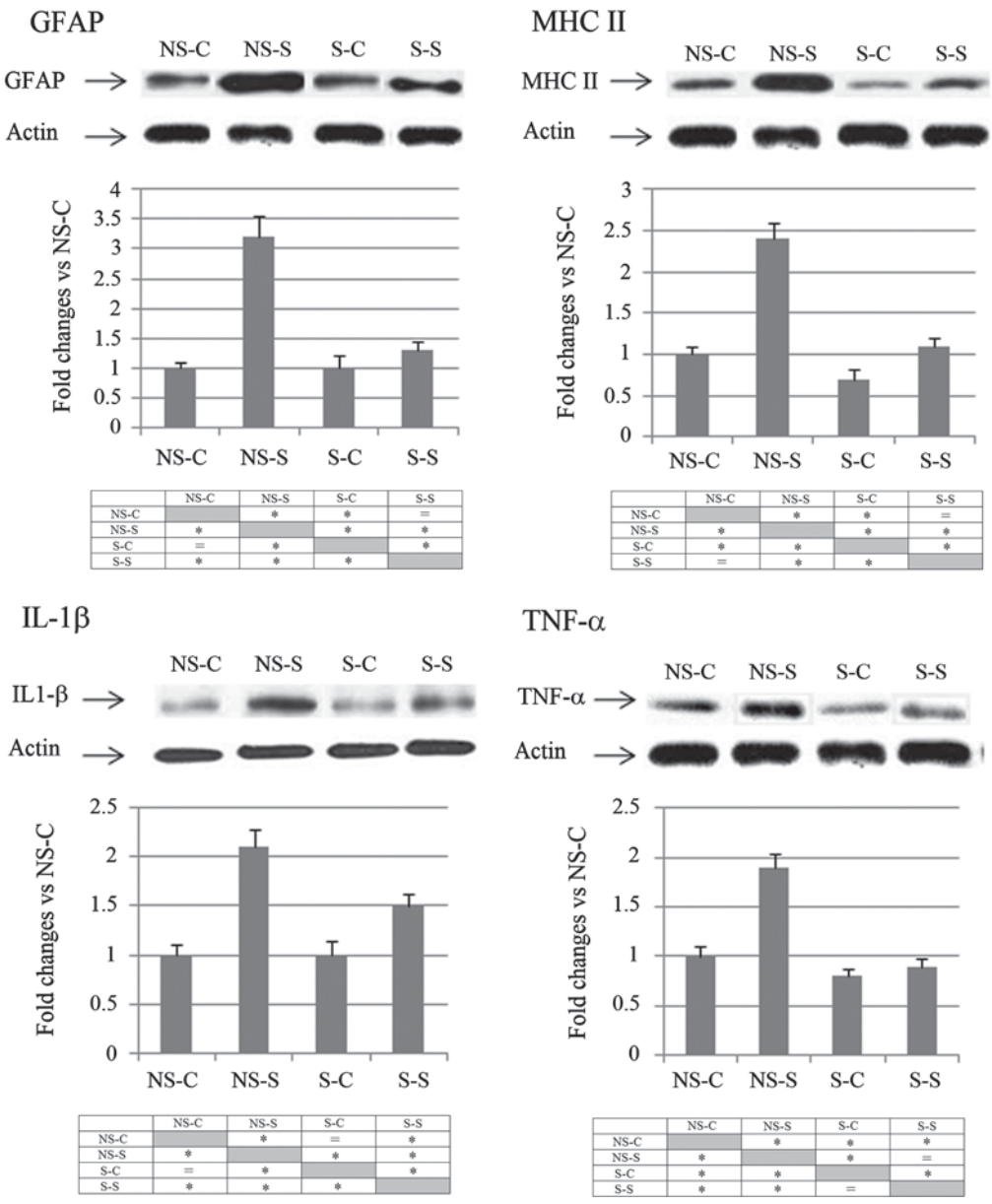

Figure 3. Modulatory effect of α-tocopherol supplementation on neuroinflammatory markers. Analysis of the expression levels of neuroinflammatory markers (GFAP, MHC II, IL-1β and TNF-α) in whole-brain protein extracts obtained from rats of different experimental groups: *NS-C*, non-supplemented sham-injected; *S-C*, supplemented sham-injected; *NS-S*, non-supplemented SE-exposed; *S-S*, supplemented SE-exposed. Per each marker protein: representative immunoblots are displayed and anti-actin blots are shown as loading control; histograms represent densitometric analyses of blots from three independent experiments (means ± SD); statistical analyses performed by one-way Anova and LSD post-hoc test are tabulated: $^{*}p < 0.05$.

staining revealed the same trend: absolute number of FJ+ events, as calculated by means of Dako CytoCount beads as previously described [29], was ~ 2.5-fold lower in S-S samples with respect to NS-S (Figure 4B; Student's *t*-test: $p < 0.001$), thus showing that neuronal degeneration was reduced by α-T supplementation.

Discussion and conclusions

Although physiological concentrations of VitE in plasma and tissues are controlled by mechanisms not fully elucidated yet, α-T appears to be transferred to peripheral tissues mainly by the delivery mechanisms of lipoprotein lipids, which include facilitated uptake by lipid transfer proteins and lipases, receptor-mediated lipoprotein endocytosis (for instance by LDL receptors) and selective lipid uptake that appear to involve SR-BI-mediated HDL uptake [30]. VitE crosses the blood–brain barrier by means of these mechanisms and its brain distribution is influenced by the regional expression of α-T transfer protein [12,31]. Other lipid binding proteins may contribute to regulate VitE bioavailability in the brain, also affecting intracellular trafficking signalling and antioxidant function of α-T [32]. Animal studies suggest that the replacement of endogenous

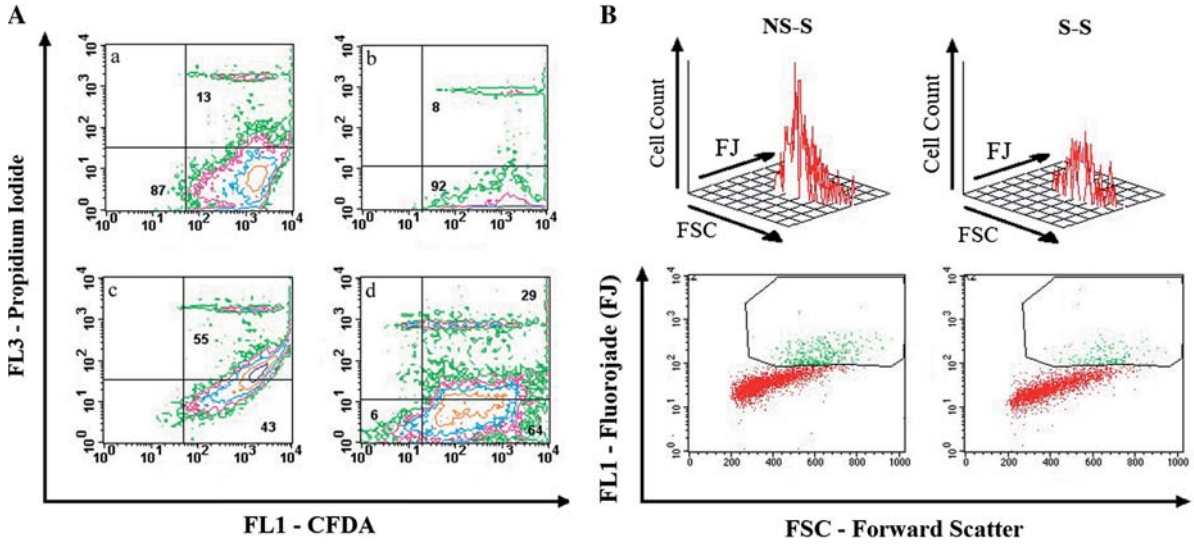

Figure 4. Flow cytometric analysis reveals the neuroprotective effect of α-tocopherol supplementation. (A) Representative contour plots FL1 (CFDA) vs FL3 (Propidium Iodide) of *S-S* (a, b) and *NS-S* (c, d) animals. Percentages of double positive CFDA/PI events (see numbers in the graphs) highlight a marked decrease of cell death in supplemented animals. (B) 3D plot (top) and dot plot (bottom) FSC vs FL1 of *NS-S* and *S-S* cells labelled with FJ probe. Both 3D and dot plot show a higher number of neurodegenerated neurons (FJ^+) in *NS-S* samples.

α-T in the brain by supplemented α-T is slower than in other tissues, such as the liver and heart [33], and this appears to be confirmed in humans [11], thus suggesting a low metabolic rate and turnover of this vitamin in brain cells. However, bioavailability and metabolism of VitE influence tissue susceptibility to lipid peroxidation, α-T being an effective fat soluble chain breaker strategically distributed in biomembranes to protect polyunsaturated fatty acid-rich phospholipids [34]. Indeed, VitE content in rat brain has been inversely associated with lipid peroxide concentrations in the dentate gyrus [35] and lipid peroxidation can be prevented by VitE supplementation in different model systems of drug-induced epilepsy [13,14].

Sustained seizure activity induces rapid increase of lipid peroxidation and disruption of mitochondrial redox status [36,37]. As shown in diverse experimental models, preliminary injections of VitE and other dietary antioxidants, such as curcumin, resveratrol and ginsenosides, reduce seizure-induced oxygen and nitrogen free radicals generation on a time scale of minutes-to-hours [14,38–41]. Here, we demonstrated that short-term dietary α-T supplementation reduces brain lipid peroxidation 4 days after KA-induced seizures. Together with a high consumption rate of brain α-T observed in supplemented rats after seizures, this finding indicates that conditions of oxidative stress initiated by SE persists long after the earliest post-ictal phases and that α-T can play a prolonged antioxidant effect after the triggering event. In non-supplemented rats, seizures provoked only a small, although significant, decrease of brain α-T, thus pointing to a minor rate of tocopherol consumption in conditions of normal VitE intake and suggesting that, under oxidative stress, the rate of brain α-T consumption varies as a function of its tissue concentrations.

In normally-fed rats, we found that GFAP and MHC II expression, as well as the levels of pro-inflammatory cytokines, were markedly increased 4 days after seizures, thus indicating that signs of astrogliosis, microglial activation and cytokines induction persist even in the absence of ongoing seizure activity. This finding is in good agreement with data in pilocarpine-induced epilepsy, reporting that early-onset inflammatory events, initiated a few hours after seizures, remain activated for days or weeks after the precipitating insult [2–4].

In vitro and *in vivo* evidence has been obtained on the role of VitE as a modulator of neuroinflammatory responses and glial cell reactivity upon exposure to various types of insults [15–19]. The present study showed that, in VitE-supplemented animals, but not in normally-fed animals, the expression of all neuroinflammatory markers decreased 4 days after seizures, providing the first evidence that dietary α-T supplementation can reduce neuroglial activation, also suppressing cytokine production in epilepsy.

A pathogenetic role of neuroinflammation in epilepsy has been proposed. Glial activation and chronically elevated levels of cytokine alter neuronal excitability by specifically interacting with receptors and ion channels [5,6]. These events enhance seizure susceptibility [8,9]. To this regard, it is important to note that in our experiments anti-inflammatory effects of α-T were assessed a few days after seizures. This is the beginning of a transition phase in which pro-inflammatory pathways remain activated favouring

disease progression, even in the absence of ongoing epileptic activity [2,21].

The mechanisms underlying anti-inflammatory effects of α-T observed in KA-induced seizures might be tightly connected with its antioxidant properties. Indeed, oxygen free radicals and peroxides, as well as reactive nitrogen species and nitric oxide, can activate redox-sensitive signalling pathways [42]. In line with this view, in both tissue cultures of murine microglia and mouse brain *in vivo*, α-T-mediated reduction of LPS-induced oxidative stress was associated with a significant inhibition of IL-6 secretion [16]. It is worth noting that a significant reduction of MHC II and TNF-α levels was also observed in supplemented rats not exposed to KA, thus showing that dietary loads of α-T may modulate microglial activation even in the absence of oxidative stress, i.e. in the presence of a normal ROS flux. This evidence may lead to speculation that, besides acting as a radical scavenger, α-T may prevent the detrimental effects of epilepsy and glial activation regulating inflammatory genes and signalling routes with mechanisms that could be partially or completely independent from the classical antioxidant function of this vitamin [20,32]; actually, different forms of VitE with similar antioxidant activity show different propensity to interact with low- and high-affinity binding proteins and receptors within the cell, which ultimately regulate signalling routes and genes associated with several responses in the brain including inflammation and cytotoxicity [20] and, as a recent example, α-T was found to exert the redox-independent down-regulation of microRNA 125b expression, which is involved in inflammation [43] as well as in astrogliosis and glial cell proliferation [44].

The present results show that, besides suppressing seizure-induced neuroglial activation, α-T markedly reduces neuronal cell death after SE. It is known that reactive microgliosis can drive progressive neurotoxicity, by initiating (and responding to) neuronal damage [45]; moreover, in epileptic models, cytokine induction precedes by several hours the appearance of degenerating neurons [46]. Collectively, these observations strongly suggest that anti-inflammatory effects of α-T supplementation can play a relevant neuroprotective role in epilepsy.

Acknowledgements

This work was supported by funds from University of Urbino 'Carlo Bo' to RC, AM and PS.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the contest and writing of the paper.

References

[1] Vezzani A, Granata T. Brain inflammation in epilepsy: experimental and clinical evidence. Epilepsia 2005;46:1724–1743.
[2] Ravizza T, Gagliardi B, Noè F, Boer K, Aronica E, Vezzani A. Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy. Neurobiol Dis 2008; 29:142–160.
[3] Shapiro LA, Wang L, Ribak CE. Rapid astrocyte and microglia activation following pilocarpine-induced seizures in rats. Epilepsia 2008;49(Suppl 2):33–41.
[4] Turrin NP, Rivest S. Innate immune reaction in response to seizures: implications for the neuropathology associated with epilepsy. Neurobiol Dis 2004;16:321–334.
[5] Schafers M, Sorkin L. Effect of cytokines on neuronal excitability. Neurosci Lett 2008;437:188–193.
[6] Vezzani A, Ravizza T, Balosso S, Aronica E. Glia as a source of cytokines: implications for neuronal excitability and survival. Epilepsia 2008;49:24–32.
[7] Auvin S, Shin D, Mazarati A, Nakagawa JA, Miyamoto J, Sankar R. Inflammation exacerbates seizure-induced injury in the immature brain. Epilepsia 2007;48:27–34.
[8] Galic MA, Riazi K, Heida JG, Mouihate A, Fournier NM, Spencer SJ, et al. Postnatal inflammation increases seizure susceptibility in adult rats. J Neurosci 2008;28:6904–6913.
[9] Somera-Molina KC, Nair S, van Eldik LJ, Watterson DM, Wainwright MS. Enhanced microglial activation and proinflammatory cytokine upregulation are linked to increased susceptibility to seizures and neurologic injury in a 'two-hit' seizure model. Brain Res 2009;1282:162–172.
[10] Kamat CD, Godal S, Mhatre M, Williamson KS, Pye QN, Hensley K. Antioxidants in central nervous system diseases: preclinical promise and translational challenges. J Alzheimers Dis 2008;15:473–493.
[11] Burton GW, Traber MG, Acuff RV, Walters DN, Kayden H, Hughes L, Ingold KU. Human plasma and tissue alpha-tocopherol concentrations in response to supplementation with use of deuterated natural and synthetic vitamin E. Am J Clin Nutr 1998;67:669–684.
[12] Gohil K, Oommen S, Quach HT, Vasu VT, Aung HH, Schock B, et al. Mice lacking alpha-tocopherol transfer protein gene have severe alpha-tocopherol deficiency in multiple regions of the central nervous system. Brain Res 2008;1201:167–176.
[13] Ribeiro MCP, de Avila DS, Schneider CYM, Hermesa FS, Furiana AF, Oliveira MS, et al. α-Tocopherol protects against pentylenetetrazol- and methylmalonate-induced convulsions. Epilepsy Res 2005;66:185–190.
[14] Tomè AR, Feng D, Freitas RM. The effects of alpha-tocopherol on hippocampal oxidative stress prior to in pilocarpine-induced seizures. Neurochem Res 2010;35:580–587.
[15] Annahazi A, Mracsko E, Sule Z, Karg E, Penke B, Bari F, Farkas E. Pre-treatment and post-treatment with alpha-tocopherol attenuates hippocampal neuronal damage in experimental cerebral hypoperfusion. Eur J Pharmacol 2007; 157:120–128.
[16] Godbout JP, Berg BM, Kelley KW, Johnson RW. α-Tocopherol reduces lipopolysaccharide-induced peroxide radical formation and interleukin-6 secretion in primary murine microglia and in brain. J Neuroimmunol 2004;149:101–109.
[17] Gonzalez-Perez O, Gonzales-Castaneda RE, Huerta M, Luquin S, Gomez-Pinedo U, Sanchez-Almaraz E, et al. Beneficial effects of α-lipoic acid plus vitamin E on neurological deficit, reactive gliosis and neuronal remodeling in the penumbra of the ischemic rat brain. Neurosci Lett 2002; 321:100–104.
[18] Li Y, Liu L, Barger SW, Mrak RE, Griffin WST. Vitamin E suppression of microglial activation is neuroprotective. J Neurosci Res 2001;66:163–170.

[19] Stolzing A, Widmer R, Jung T, Voss P, Grune T. Tocopherol-mediated modulation of age-related changes in microglial cells: turnover of extracellular oxidized protein material. Free Rad Biol Med 2006;40:2126–2135.
[20] Galli F, Azzi A. Present trends in vitamin E research. Biofactors 2010;36:33–42.
[21] Pitkanen A, Sutula TP. Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. Lancet Neurol 2002;1:173–181.
[22] Sperk G. Kainic acid seizures in the rat. Prog Neurobiol 1994;42:1–32.
[23] Betti M, Ambrogini P, Minelli A, Floridi A, Lattanzi D, Ciuffoli S, et al. Maternal dietary loads of alpha-tocopherol depress protein kinase C signaling and synaptic plasticity in rat postnatal developing hippocampus and promote permanent deficits in adult offspring. J Nutr Biochem 2011;22:60–70.
[24] Campanella M, Sciorati C, Tarozzo G, Beltramo M. Flow cytometric analysis of inflammatory cells in ischemic rat brain. Stroke 2002;33:586–592.
[25] Canonico B, Betti M, Luchetti F, Battistelli M, Falcieri E, Ferri P, et al. Flow cytometric profiles, biomolecular and morpohological aspects of transfixed leukocytes and red cells. Cytometry B Clin Cytom 2010;78:267–278.
[26] Massaro EJ, Elstein KH, Zucker RM, Bair KW. Limitations of the fluorescent probe viability assay. Mol Toxicol 1989;2:271–284.
[27] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–358.
[28] Meydani M, Lipman RD, Han SN, Wu D, Beharka A, Martin KR, et al. The effect of long-term dietary supplementation with antioxidants. Ann NY Acad Sci 1998;854:352–360.
[29] Canonico B, Zamai L, Burattini S, Granger V, Mannello F, Gobbi P, et al. Evaluation of leukocyte stabilisation in TransFix-treated blood samples by flow cytometry and transmission electron microscopy. J Immunol Methods 2004;295:67–78.
[30] Mardones P, Rigotti A. Cellular mechanisms of vitamin E uptake: relevance in a-tocopherol metabolism and potential implications for disease. J Nutr Biochem 2004;15:252–260.
[31] Yokota T, Igarashi K, Uchihara T, Jishage K, Tomita H, Inaba A, et al. Delayed-onset ataxia in mice lacking alpha -tocopherol transfer protein: model for neuronal degeneration caused by chronic oxidative stress. Proc Natl Acad Sci USA 2001;98:15185–15190.
[32] Brigelius-Flohè R. Vitamin E: the shrew waiting to be tamed. Free Rad Biol Med 2009;46:543–554.
[33] Nishio K, Ishida N, Saito Y, Ogawa-Akazawa Y, Shichiri M, Yoshida Y, et al. α-Tocopheryl phosphate: uptake, hydrolysis, and antioxidant action in cultured cells and mouse. Free Radic Biol Med 2011;50:1974–1980.
[34] Atkinson J, Harroun T, Wassall SR, Stillwell W, Katsaras J. The location and behavior of a-tocopherol in membranes. Mol Nutr Food Res 2010;54:1–11.
[35] Murray CA, Lynch MA. Dietary supplementation with vitamin E reverses the age-related deficit in long term potentiation in dentate gyrus. J Biol Chem 1998;273:12161–12168.
[36] Milatovic D, Gupta RC, Dettbarn WD. Involvement of nitric oxide in kainic acid-induced excitotoxicity in rat brain. Brain Res 2002;957:330–337.
[37] Waldbaum S, Patel M. Mitochondria, oxidative stress, and temporal lobe epilepsy. Epilepsy Res 2010;88:23–45.
[38] Gupta YK, Briyal S, Chaudhary G. Protective effect of trans-resveratrol against kainic acid-induced seizures and oxidative stress in rats. Pharmacol Biochem Behav 2002;71:245–249.
[39] Gupta YK, Briyal S, Sharma M. Protective effect of curcumin against kainic acid-induced seizures and oxidative stress in rats. Indian J Physiol Pharmacol 2009;53:39–46.
[40] Shin EG, Jeong JH, Kim AY, Koh YH, Nah SY, Kim WK, et al. Protection against kainate neurotoxicity by gingenosides: attenuation of convulsive behavior, mitochondrial dysfunction, and oxidative stress. J Neurosci Res 2009;87:710–722.
[41] Zaja-Milatovic S, Gupta RC, Aschner M, Montine TJ, Milatovic D. Pharmacological suppression of oxidative damage and dendritic degeneration following kainic acid-induced excitotoxicity in mouse cerebrum. Neurotoxicology 2008;29:621–627.
[42] Wang JY, Wen LL, Huang YN, Chen YT, Ku MC. Dual effects of antioxidants in neurodegeneration: direct neuroprotection against oxidative stress and indirect protection via suppression of glia-mediated inflammation. Curr Pharmacol Des 2006;12:3521–3533.
[43] Rimbach G, Moehring J, Huebbe P, Lodge JK. Gene-regulatory activity of alpha-tocopherol. Molecules 2010;15:1746–1761.
[44] Pogue AI, Cui JG, Li YY, Zhao Y, Culicchia F, Lukiw WJ. Micro RNA-125b (miRNA-125b) function in astrogliosis and glial cell proliferation. Neurosci Lett 2010;476:18–22.
[45] Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 2007;8:57–69.
[46] Ravizza T, Rizzi M, Perego C, Richichi C, Veliskova J, Moshè SL, et al. Inflammatory response and glia activation in developing rat hippocampus after status epilepticus. Epilepsia 2005;46:113–117.

This paper was first published online on Early Online on 12 July 2011.