

Vitamin E reduces amyloidosis and improves cognitive function in Tg2576 mice following repetitive concussive brain injury

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Abstract

Traumatic brain injury is a well-recognized environmental risk factor for developing Alzheimer's disease. Repetitive concussive brain injury (RCBI) exacerbates brain lipid peroxidation, accelerates amyloid (A β) formation and deposition, as well as cognitive impairments in Tg2576 mice. This study evaluated the effects of vitamin E on these four parameters in Tg2576 mice following RCBI. Eleven-month-old mice were randomized to receive either regular chow or chow-supplemented with vitamin E for 4 weeks, and subjected to RCBI (two injuries, 24 h apart) using a modified controlled cortical impact model of closed head injury. The same dietary regimens were maintained up to 8 weeks post-injury, when the animals were killed for biochemical and immunohistochemical

analyses after behavioral evaluation. Vitamin E-treated animals showed a significant increase in brain vitamin E levels and a significant decrease in brain lipid peroxidation levels. After RCBI, compared with the group on regular chow, animals receiving vitamin E did not show the increase in A β peptides, and had a significant attenuation of learning deficits. This study suggests that the exacerbation of brain oxidative stress following RCBI plays a mechanistic role in accelerating A β accumulation and behavioral impairments in the Tg2576 mice.

Keywords: Alzheimer's disease, amyloidosis, head trauma, oxidative stress, vitamin E.

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Alzheimer's disease (AD) is the most common form of dementia in the elderly (Ritchie and Lovestone 2002). It is characterized by two types of protein aggregates, neurofibrillary tangles and amyloid (A β) plaques, mainly distributed in brain areas that are involved in learning and memory. The most common form of AD is sporadic AD (SAD), accounting for more than 90% of all disease cases. While apolipoprotein E ϵ 4 allele is the best-documented genetic risk factor for SAD (Corder *et al.* 1993; Saunders *et al.* 1993; Strittmatter *et al.* 1993), traumatic brain injury (TBI) represents the most robust environmental AD risk factor (Schofield *et al.* 1997; Nemetz *et al.* 1999; Plassman *et al.* 2000; Jellinger *et al.* 2001). TBI has been shown to induce an

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Abbreviations used: A β , amyloid beta; AD, Alzheimer's disease; APPswe, A β precursor protein; BSA, bovine serum albumin; CCI, controlled cortical impact; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; HP, hippocampus; HPLC, high-performance liquid chromatography; IHC, immunohistochemistry; iPs, isoprostanes; LPO, lipid peroxidation; MWM, Morris water maze; PBS, phosphate-buffered saline; PHC, peri-hippocampal cortex; RCBI, repetitive concussive brain injury; SAD, sporadic Alzheimer's disease; SSC, somatosensory cortex; TBI, traumatic brain injury.

increase in cerebrospinal fluid (CSF) A β levels (Raby *et al.* 1998), as well as A β deposition in the human brain (Nicoll *et al.* 1995), and A β plaques have been found within days after a single incident of TBI in humans (Roberts *et al.* 1991; Graham *et al.* 1995). However, recent conflicting results have also been reported (Franz *et al.* 2003; Kay *et al.* 2003).

Formation of reactive oxygen species and subsequent oxidative damage has been extensively shown in TBI (Lewen *et al.* 2000; Marklund *et al.* 2001). Oxidative damage to the CNS predominantly manifests as lipid peroxidation (LPO) because of the high content of polyunsaturated fatty acids in the brain that are particularly susceptible to oxidation. Isoprostanes (iPs) are specific and sensitive markers of LPO *in vivo* (Praticò *et al.* 2001a) and have been shown to increase following experimental TBI in rats (Praticò *et al.* 2002). Further, we have shown that brain LPO correlates with amyloid plaque formation and deposition in the Tg2576 mice, a mouse model of AD-like brain amyloidosis which overexpresses mutant human A β precursor protein (APPswe; Hsiao *et al.* 1996; Praticò *et al.* 2001b).

Previously, using this mouse model we have provided experimental evidence in support of a linkage between TBI and AD, showing that repetitive concussive brain injury (RCBI) accelerates brain A β accumulation and behavioral impairments, which were coincident with an exacerbation of brain LPO (Uryu *et al.* 2002). In the present study, we took a step further and investigated whether in this model exacerbation of the oxidative stress response is mechanistically linked to the acceleration of the AD-like phenotype in the Tg2576 mice undergoing RCBI. For this purpose, we randomized Tg2576 mice to either receive regular chow or chow supplemented with a potent exogenous antioxidant, vitamin E, and we subjected them to RCBI. Here, we report that vitamin E, by suppressing the exacerbation of brain LPO following RCBI, prevents A β peptide accelerated formation and behavioral deficits in these mice. These findings support the hypothesis that increased oxidative stress response plays a functional role in the accelerated amyloidogenic response after RCBI, and that antioxidant therapies could decrease the risk of AD associated with previous episodes of brain trauma.

Materials and methods

Animals and surgical procedure

All the procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania approved them.

At 11 months of age, Tg2576 female mice were randomized to receive either a regular chow or chow supplemented with 2 IU/g diet vitamin E. The choice of using only female mice was based on

previous work showing that in this animal model females exhibit a more consistent and greater amount of A β formation and deposition than males (Callahan *et al.* 2001). Preliminary experiments demonstrated that the selected dose of vitamin E significantly reduces *in vivo* LPO (Praticò *et al.* 1998a). The animals were kept in the same dietary regimen for all the duration of the study. At 12 months of age, Tg2576 mice were subjected to concussive brain injury (CBI) repetitively (RCBI) as described previously (Laurer *et al.* 2001). Briefly, mice were anesthetized with inhalation anesthesia via a nose cone using 2% isoflurane; ointment was applied to their eyes to protect corneas during surgery. The mice were kept on a heating pad to maintain body temperature throughout the surgical procedures. All animals were mounted in a stereotactic frame; a skin incision was performed to expose the skull; and the mice remained in the stereotactic apparatus while subjected to RCBI using a pressure-driven instrument that is mechanically identical to a previously described controlled cortical impact device (Dixon *et al.* 1991; Smith *et al.* 1995) with minor modifications previously described (Laurer *et al.* 2001). In order to obtain a concussive injury, which better recapitulates the features of a diffuse injury, we replaced the standard tip with a less rigid and with larger diameter (9 mm) silicone impounder. Using a larger, more compliant tip provided a method to distribute impact energy over a larger surface of the brain and also allowed deformation to encompass more of the brain. The impounder was rigidly mounted at an angle of 20° from vertical, the depth and duration of the impact were kept constant, and head movements were minimal while delivering the load. The impounder was driven at 4.8–5.0 m/s to a depth of 2.5 mm lower than the zero point, causing a non-penetrating concussive blow to the head. The procedure was completed with the closure of the incision using 4-0 silk sutures. The animals were removed from the stereotactic frame and placed in a heated cage, and after recovery from anesthesia (as evidenced by ambulation), they were returned to their home cages. Twenty-four hours after the first CBI, the animals were anesthetized as described above and subjected to a second CBI in the same location over the left parietotemporal region. Sham-treated animals also were anesthetized and placed in the stereotactic frame; the incision was sutured closed without brain injury on two consecutive days thereby following exactly the surgical procedures of repetitive CBI.

Neurobehavioral analysis

The testing paradigm for evaluation of cognitive function using the Morris water maze (MWM) has been described in detail previously (Smith *et al.* 1991, 1995). Briefly, the MWM is a circular pool 1 m in diameter, painted white inside (Morris *et al.* 1982). The water (18–22°C) is made opaque by adding non-toxic, water-soluble white coloring. To test for RCBI-induced learning impairments, animals received no training in the MWM before injury and were tested for their ability in locating a stationary, submerged platform (0.5 cm below the surface) using external clues within a maximum of 60 s for each trial, during four trials/day over an 8-day period starting at 8 weeks after injury. The essential feature of the MWM is that mice can escape from the water onto the platform after being placed randomly at one of four sites in the pool. Latencies of four trials/day were recorded and averaged to obtain a measurement for the performance of each animal on a given day. Analysis was always performed in a coded fashion. The composite neuroscore, which

includes a battery of motor tests, was obtained for all mice before performing MWM evaluations as previously described (Uryu *et al.* 2002). Each animal was scored by an investigator blinded to the injury status or treatment of the animals.

Histological and immunohistochemical analysis

Eight weeks post-injury mice were anesthetized (sodium pentobarbital i.p., 65 mg/kg) and then transcardially perfused with phosphate-buffered saline (PBS; 0.1 M, pH 7.4) followed by 10% neutral buffered formalin. The brains were removed, post-fixed overnight, sliced into 2-mm-thick coronal slabs, and embedded in paraffin in a frontal to occipital series of blocks, and the blocks were cut in a near serial array of 6- μ m thick coronal sections. Paraffin sections were subjected to immunohistochemistry (IHC) as previously described (Murai *et al.* 1998; Nakagawa *et al.* 1999, 2000). A β deposits were visualized by NAB228, A β monoclonal antibody (mAb; Lee *et al.* 2003). Our preliminary study revealed that NAB228 and 4G8 showed identical staining patterns. Briefly, sections were deparaffinized in xylene and hydrated in a series of ethanol, subsequently subjected to treatment with formic acid (88%; 20 min), methanol (150 mL)-hydrogen peroxide (33% 30 mL; 30 min), and fetal bovine serum (4%) in Tris-buffer (0.1 M, pH 7.4) before applying the primary antibody NAB228 (1 : 10 000 dilution) at 4°C. The sections were incubated with the primary antibody overnight (17–20 h). Subsequently, the biotinylated anti-mouse IgG and the avidin–biotin complex method were used according to the instructions of the vendor (Vector Laboratories, Burlingame, CA, USA). Negative controls included the application of the exact same IHC protocol, except pre-immune serum was applied instead of primary antibody.

Image analysis

For the quantitative analysis, seven to eight sections from each animal between –1.2 and –1.8 relative to bregma (Franklin and Paxinos 1997) were always analyzed in a coded fashion and altogether in a single experiment. They were stained with NAB228 antibody and not counterstained. The quantitative image analysis was carried out as described previously with minor modifications (Uryu *et al.* 2002). Light microscopic images from the somatosensory cortex (SSC), peri-hippocampal cortex (PHC), and hippocampus (HP) from both ipsilateral (left) and contralateral (right) hemispheres to the RCBI site were captured from eight series of sections using a Nikon Microphot-FXA microscope with a 4 \times objective lens. Using a personal computer, each image was opened with image analysis software (Image Pro-plus; Media Cybernetics, Inc., Silver Spring, MD, USA). Manual editing was then performed to eliminate non-specific signals (e.g. blood vessels and staining artifacts). The areas occupied by A β -immunoreactive products in the regions of interest were measured, and the total area occupied by the outlined structures was measured to calculate:

1. the total area with selected immunoreactive products;
 2. the percentage of the area occupied by immunoreactive products over the outlined anatomical area in the image.
- The analysis was always performed in a coded fashion.

Sandwich A β ELISA

For quantification of A β brain levels, brains were removed, and both right and left cerebral cortex, hippocampus, and cerebellum were

separated, collected in individual test tubes, weighed, and immediately frozen until analysis. Sequential extraction of samples was performed with high salt buffer and formic acid to measure soluble and insoluble brain A β (x-40) and A β (x-42/43) levels. Cerebral cortices, hippocampus, and cerebellum homogenates were serially extracted in high salt reassembly buffer (0.1 M Tris, 1 mM EGTA, 0.5 mM MgSO₄, 0.75 M NaCl, and 0.02 M NaF, pH 7.0) containing a protease inhibitor mixture (pepstatin A, leupeptin, *N*-tosyl-L-phenylalanine chloromethyl ketone, *N* α -p-tosyl-L-lysine chloromethyl ketone, and soybean trypsin inhibitor, each at 1 μ g/mL in 5 mM EDTA). Homogenates were centrifuged at 100 000 g for 1 h at 4°C. Supernatants were removed, and pellets re-suspended in 70% formic acid, and re-sonicated and centrifuged at 100 000 g for 1 h at 4°C. Supernatants were removed and diluted 1 : 20 with 1 M Tris base. Extracted samples were normalized to their original wet weight and analyzed separately by enzyme-linked immunosorbent assay (ELISA). To do this, they were diluted in buffer EC [0.02 M sodium phosphate, 0.2 M EDTA, 0.4 M NaCl, 0.2% bovine serum albumin (BSA), 0.05% 3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulfonic acid, 0.4% Block-ace (Dainippon; Suita, Osaka, Japan), and 0.05% sodium azide, pH 7.0] and analyzed using Ban50 as the capturing mAb and BA-27 and BC-05 mAbs to detect A β 1–40 and A β 1–42, respectively, as described previously (Wang *et al.* 1999; Nakagawa *et al.* 2000; Uryu *et al.* 2002). The analysis was always performed in a coded fashion.

Quantification of vitamin E and isoprostane

Aliquots of brain tissue homogenates were used for iPs and vitamin E analysis. Briefly, vitamin E levels were assayed by high-performance liquid chromatography (HPLC; Praticò *et al.* 1998a), while 8,12-*iso*-iPF_{2 α} -VI by a standardized gas chromatography/mass spectrometry assay, as previously described (Praticò *et al.* 2001b). All the assays were always performed without knowledge of the treatment group of the mice.

Statistical analysis

Levels of vitamin E, 8,12-*iso*-iPF_{2 α} -VI, A β 1–40 and A β 1–42 concentrations and A β deposits were expressed as mean \pm SD. Data from left and right hemispheres were compared by paired *t*-test. Averages of the two hemispheres were compared by unpaired *t*-test. Data obtained in the MWM are parametric and are given as mean \pm SD. These data were analyzed using a one-way ANOVA for repeated measures (learning effect, treatment effect). Significance was always set at *p* < 0.05.

Results

Brain levels of vitamin E and 8,12-*iso*-iPF_{2 α} -VI isoprostane

Starting at 11 months of age, Tg2576 mice were randomized to receive normal chow (*n* = 13) or chow supplemented with vitamin E (2 IU/g diet; *n* = 9). After 4 weeks, mice were subjected to RCBI and kept on the same diets for an additional 8 weeks, then killed. At this time, the treated group had vitamin E plasma levels significantly higher (*p* < 0.01) than the control group (50.6 \pm 4.2 μ M and

$20.8 \pm 3.9 \mu\text{M}$, respectively). Levels of brain vitamin E and 8,12-*iso*-iPF_{2α}-VI obtained from both cortex and hippocampus homogenates were also measured. Differences between right and left side were not found and values obtained were averaged for both hippocampus and cortex. In both regions, while no difference was observed between sham-operated and the RCBI-group on regular chow (18 ± 2 vs. 21 ± 2.5 pg/mg tissue), vitamin E brain levels increased in the RCBI-treated mice on vitamin E-supplemented diet (38 ± 3.5 pg/mg tissue, $p < 0.01$). Compared with the sham-treated mice, mice undergoing RCBI showed in the same regions a significant increase in 8,12-*iso*-iPF_{2α}-VI levels, which was prevented in the vitamin E group ($p < 0.01$; Fig. 1).

Neurobehavioral analyses

When we tested the motor function of all mice, we did not observe any sign of functional impairment (not shown). Confirming our previous study (Uryu *et al.* 2002), we observed that compared with sham-operated, Tg2576 mice subjected to RCBI manifest a significant cognitive impairment in the MWM test. However, RCBI-treated Tg2576 animals fed vitamin E-supplemented diet ($n = 8$) demonstrated a better ability in learning to locate the platform than mice on regular chow ($n = 10$; RM ANOVA $p < 0.01$; Fig. 2).

Aβ ELISA

Next, we tested whether or not vitamin E modulates accumulation of Aβ peptides in the brain following RCBI by a sensitive sandwich ELISA analysis. We measured levels of Aβ1–40 and Aβ1–42 in the high salt and formic acid soluble fractions from cortex and hippocampus homogenates. Confirming previous results (Uryu *et al.* 2002), there was no difference between the ipsilateral side and contralateral side to injury in the brain from normal diet group ($p = 0.81$), as well as that from vitamin E diet group ($p = 0.70$). Similarly, we found that, compared with the sham group, RCBI-treated mice showed an increase in both Aβ1–40 (4.8 ± 1.8 vs. 8 ± 2 pmol/g tissue, $p < 0.05$) and Aβ1–42 (36 ± 8 vs. 48 ± 6 pmol/g tissue, $p < 0.05$). However, brains from the

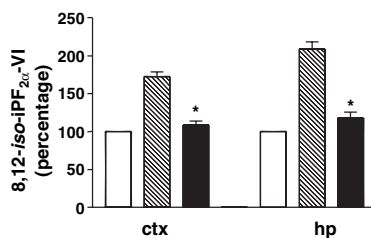


Fig. 1 Levels of 8,12-*iso*-iPF_{2α}-VI in cortical (ctx) and hippocampal (hp) homogenates from sham-operated (open bars) or RCBI-treated Tg2576 on regular chow (hatched bar), or vitamin E (closed bar), results are expressed as mean \pm SD (* $p < 0.01$).

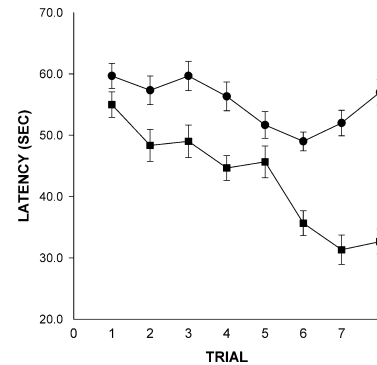


Fig. 2 Data from MWM testing from Tg2576 mice receiving regular chow (●) or vitamin E (■). Trials were made in consecutive 8-day sessions at 8 weeks after injury (one-way RM ANOVA, learning over time: $p < 0.01$, treatment effect: $p < 0.01$).

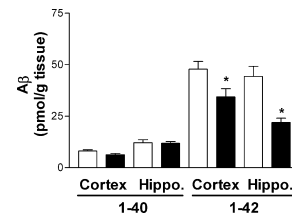


Fig. 3 Levels of high salt soluble Aβ 1–40 and Aβ 1–42 in cortex and hippocampus (hippo) homogenates from Tg2576 receiving regular chow ($n = 7$; □) or vitamin E ($n = 6$; ■). Results are expressed as mean \pm SD (* $p < 0.01$).

RCBI-treated mice receiving vitamin E showed a significant reduction in Aβ1–42 in both high salt and formic acid soluble fractions in the cortex and hippocampus (Figs 3 and 4) compared to the ones on regular chow. Furthermore, while we did not observe an effect in the cortex levels of formic acid-soluble Aβ1–40, hippocampal levels were lower in vitamin E group compared to the control group ($p < 0.01$; Fig. 4)

Amyloid deposition

Eight weeks after RCBI, all analyzed brains showed various amounts of Aβ deposition widely distributed in the cerebral cortex and hippocampus as shown by the pan-Aβ antibody, NAB228. Confirming previous results (Uryu *et al.* 2002), we observed that 8 weeks after RCBI there was no significant difference in amyloid deposition between sham- and RCBI-treated on regular chow (not shown). Further, we observed that the distribution pattern of Aβ deposition was infrequent and uneven whether or not the RCBI-treated mice were on the normal diet ($n = 5$) or vitamin E-supplemented diet ($n = 4$). To determine whether or not vitamin E had an effect on amyloid deposition following RCBI, the area occupied by Aβ-immunoreactive deposits in the SSC, PHC, and HP both ipsilateral and contralateral hemisphere to the impact site

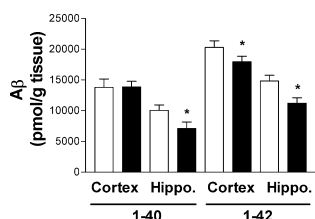


Fig. 4 Levels of formic acid soluble A β 1–40 and A β 1–42 in cortex and hippocampus (hippo) homogenates from Tg2576 receiving regular chow ($n = 7$; \square) or vitamin E ($n = 6$; \blacksquare). Results are expressed as mean \pm SD (* $p < 0.01$).

were analyzed. First, there was no significant difference in the amyloid burden between injured and contralateral side (SSC, $p = 0.41$; HP, $p = 0.26$; PHC, $p = 0.32$) of the brains of mice undergoing RCBI. Second, while there was a trend toward reduction in A β burden in the vitamin E diet group when compared to the normal diet group, it did not reach statistical significance (SSC, $p = 0.17$; HP, $p = 0.14$; PHC, $p = 0.18$).

Discussion

The present study shows that in the Tg2576 mice undergoing RCBI vitamin E treatment by reducing the exacerbation of brain LPO, prevents A β peptides accumulation and behavioral deficits. These findings suggest that, in this animal model, RCBI-dependent increase in brain oxidative stress plays a functional role in accelerating of the AD-like phenotype.

Despite the fact that epidemiological studies have implicated recurrent brain trauma as a potent environmental risk factor for developing AD, the mechanism(s) responsible for this have yet to be completely elucidated (Nandoe *et al.* 2002). Thus, for years the relationship between experimental TBI and brain amyloidosis has been characterized by conflicting results. Reports have shown that following controlled cortical impact (CCI), the APP-YAC mice do not develop plaques when evaluated acutely after injury (Murai *et al.* 1998), and in PD-APP mice, CCI produces a marked ipsilateral hippocampal atrophy, almost complete CA3-CA2 cell loss and diminished A β deposition during aging (Smith *et al.* 1998; Nakagawa *et al.* 1999, 2000). On the contrary, in PD-APP mice subjected to CCI brain injury, amyloid deposition is accelerated only in the presence of APO-E4 while cell loss is not influenced (Hartman *et al.* 2002).

Recently, we have developed a new mild TBI mouse model that does not require craniotomy, produces minimal structural brain damage, and induces a progressive cell dysfunction instead of cell loss (Laurer *et al.* 2001; Uryu *et al.* 2002). This model more closely mimics the type of human injuries that predispose individuals who survive

episodes of TBI to develop AD later in life. This experimental paradigm has emerged as a more appropriate model than CCI to study the relationship between TBI and the development of amyloidosis. Previously, we have shown that, in Tg2576 mice, RCBI induces an exacerbation of brain LPO which is coincidental with an acceleration in A β formation and deposition, and cognitive impairments (Uryu *et al.* 2002). In the present study, we confirmed this observation and provided strong evidence supporting the hypothesis that exacerbation of the brain oxidative stress response secondary to the experimental head traumatic injury is the responsible factor for the acceleration of the AD-like phenotype in Tg2576 mice undergoing RCBI.

For a long time it has been known that reactive oxygen species and oxidative stress are constant features of the post-TBI syndrome, however, the functional role of this phenomena has been less clear (Hall *et al.* 1993; Shohami *et al.* 1997; Marklund *et al.* 2001). In the last 10 years the presence of signature markers of oxidative stress has been widely described for AD brains (Smith *et al.* 2000). Recent evidence from animal models and human studies suggest the novel concept that oxidative stress is an early and functional event in AD pathogenesis (Nunomura *et al.* 2001; Praticò 2002). Interestingly, in our previous study, both single and repetitive mild TBI exacerbated brain LPO in Tg2576, but single mild TBI to a lesser extent than repetitive, and the latter was associated with a more significant increase in the amyloidogenic response (Uryu *et al.* 2002).

Isoprostanes (iPs) are chemically stable isomers of prostaglandins; they are formed by a free radical peroxidation of polyunsaturated fatty acids and are sensitive and specific markers of LPO *in vivo* (Praticò *et al.* 2001a). They are selectively increased in affected regions of AD brains as well as in CSF of patients with AD (Praticò *et al.* 1998b, 2000; Montine *et al.* 1999), and correlate with the amount of amyloid deposited in the Tg2576 (Praticò *et al.* 2001b).

Vitamin E is the most important lipid-soluble chain-breaking natural antioxidant in mammalian cells (Praticò 2001). In the past 20 years, many studies have conclusively demonstrated that its principal function is to defend tissue against oxidative damage, although other biological properties have also been described (Parks and Traber 2000). Previous studies have shown that vitamin E is able to cross the blood–brain barrier and, with time, accumulate at therapeutic levels in the CNS, where it significantly reduces lipid peroxidation (Veinbergs *et al.* 2000). Moreover, we have shown that high-dose vitamin E is effective in modulating amyloid levels and deposition in Tg2576 only when administered at an early stage of its phenotype (Sung *et al.* 2004). Based on previous published work, in the current study, vitamin E was given at the same concentration and, most importantly, at an age that coincides with the initial phase of the amyloid deposition (11 months; Kawarabayashi *et al.* 2001; Praticò *et al.* 2001b). In this experimental

setting, vitamin E significantly prevented the accelerated A β accumulation by reducing the exacerbation of brain LPO secondary to the brain trauma, and improved behavioral impairments in the Tg2576 that follow RCBI. This finding supports a functional role for oxidative stress in regulating the amyloidogenic response to brain trauma *in vivo*, and it is consistent with some *in vitro* data showing that oxidative stress can positively modulate APP metabolism and increase in A β formation (Misonou *et al.* 2000). Thus, previous studies have shown that oxidants can indeed increase BACE activity and amyloid formation in neuronal cell lines (Tamagno *et al.* 2002).

Further, it is known that the levels of brain A β are the result of a balance between the amount produced in the CNS with the amount cleared from the brain into the circulation (DeMattos *et al.* 2002). Because in the present study we did not measure circulating levels of A β , we cannot exclude that vitamin E effect was also mediated by this mechanism.

In the present study, despite a trend towards reduction, we did not find a significant effect of vitamin E treatment on plaque deposition. This is because the mice were analyzed at a relatively short time after RCBI (8 weeks), and we have already shown that only after 16 weeks following RCBI there is a significant effect on plaque load (Uryu *et al.* 2002). Another possible explanation is the small number of mice analyzed together with the variability in plaque deposition that characterizes this model (Hsiao *et al.* 1996).

In summary, our data support the hypothesis that TBI accelerates the development of the AD-like phenotype in the Tg2576 by exacerbating brain oxidative stress responses. We conclude that antioxidant therapies should be undertaken at the earliest possible time in individuals who survive an episode of TBI in order to reduce the higher risk of developing AD, which has been reported in these individuals.

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