JOURNAL OF NEUROTRAUMA 24:1587–1595 (October 2007)

DOI: 10.1089/neu.2007.0313

Omega-3 Fatty Acids Supplementation Restores Mechanisms that Maintain Brain Homeostasis in Traumatic Brain Injury

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ABSTRACT

Traumatic brain injury (TBI) produces a state of vulnerability that reduces the brain capacity to cope with secondary insults. The silent information regulator 2 (Sir2) has been implicated with maintaining genomic stability and cellular homeostasis under challenging situation. Here we explore the possibility that the action of Sir2α (mammalian Sir2) in the brain can extend to serve neuronal plasticity. We provide novel evidence showing that mild TBI reduces the expression of Sir2α in the hippocampus, in proportion to increased levels of protein oxidation. In addition, we show that dietary supplementation of omega-3 fatty acids that ameliorates protein oxidation was effective to reverse the reduction of Sir2α level in injured rats. Given that oxidative stress is a subproduct of dysfunctional energy homeostasis, we measured AMP-activated protein kinase (AMPK) and phosphorylated-AMPK (p-AMPK) to have an indication of the energy status of cells. Hippocampal levels of total and phosphorylated AMPK were reduced after TBI and levels were normalized by omega-3 fatty acts supplements. Further, we found that TBI reduced ubiquitous mitochondrial creatine kinase (uMtCK), an enzyme implicated in the energetic regulation of Ca^{2+}-pumps and in the maintenance of Ca^{2+}-homeostasis. Omega-3 fatty acids supplements normalized the levels of uMtCK after lesion. Furthermore, we found that the correlation between Sir2α and AMPK or p-AMPK was disrupted by TBI, but restored by omega-3 fatty acids supplements. Our results suggest that TBI may compromise neuronal protective mechanisms by involving the action of Sir2α. In addition, results show the capacity of omega-3 fatty acids to counteract some of the effects of TBI by normalizing levels of molecular systems associated with energy homeostasis.

Key words: hippocampus; omega-3 fatty acids; oxidative stress; Sir2α

INTRODUCTION

TRAUMATIC BRAIN INJURY (TBI) is associated with a long-lasting decrement in the capacity of the brain to cope with subsequent insults and often with impairment in cognitive abilities. It still remains to be understood what are the molecular events responsible for deteriorated plasticity after TBI. It is becoming well accepted that TBI reduces metabolic energy, and that this energy deficiency can compromise neuronal function and plasticity. This implies that molecular systems closely associated with energy metabolism such as the silent information regulator 2 (Sir2) can be primary targets for the effects of TBI. Sir2 is a nicotinamide adenine dinu-

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cleotide (NAD)–dependent protein deacetylase that has been implicated in cellular homeostasis and energy metabolism (i.e., fatty acid and glucose metabolism) (Starai et al., 2002; Hallows et al., 2006). Sir2 was originally identified by its role in mediating longevity in yeast in a process largely dependent on energy homeostasis (Lin et al. 2000, 2004; Anderson et al., 2003; Rogina and Helfand, 2004). We have recently found that oxidative stress, a byproduct of dysfunctional energy metabolism, reduces levels of Sir2 in the brain. These results suggest the possibility that select molecular systems such as Sir2, acting at the interface between energy metabolism and neuronal plasticity, can convey the effects of brain challenges into long-term brain plasticity.

According to recent studies, Sir2 may play an important role in mechanisms that provide neuroprotection in mammals. Deletion of Sir2 gene has been reported to cause developmental deficits in the brain, heart, and retina in mice (Cheng et al., 2003; Sakamoto et al., 2004). It has been suggested that Sir2α can provide brain protection under challenging conditions by enhancing cellular resistance to stress and DNA repair (Araki et al., 2004; Bordone and Guarente et al., 2005; Parker et al., 2005; Sinclair, 2005). Insults to the brain associated with lifestyle such as consumption of a diet with harmful consequences for synaptic plasticity reduces the expression of Sir2α in the hippocampus and cerebral cortex (Wu et al., 2006). These findings raise the possibility that Sir2 may provide a link between the effects of lifestyle and molecular mechanisms that provide long-term genomic stability and that can provide resistant to insults. Therefore, in the present study we have investigated the effects of induced TBI on hippocampal levels of Sir2 and the possibility that a good diet rich in omega-3 fatty acids can modulate Sir2 levels. A fish oil diet rich in omega-3 fatty acids has been shown to protect the brain against the effects of TBI (Wu et al., 2004). The mechanisms by which the omega-3 fatty acids can counteract the effects of TBI are not well understood. It has been shown that elevated oxidative stress, a prevalent feature of TBI, can reduce the production of Sir2 (Wu et al., 2006), while a diet rich in omega 3 fatty acids has antioxidant capacity (Wu et al., 2004). Hence, we have embarked in studies to determine the capacity of the omega-3 fatty acids dietary supplementation to counteract a decrease in Sir2α and maintain neuronal homeostasis after TBI.

METHODS

Experimental Designs and Tissue Preparation

Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) weighing 200–240 g were housed in cages and maintained in environmentally controlled rooms (22–24°C) with a 12 h light/dark cycle. After acclimatization for 1 week on standard rat chow, one set of rats were exposed to mild fluid percussion injury (FPI) for determining the effects of TBI at different time point post-lesion. After 1, 7, or 14 days post-injury, the rats (n = 5–6 within each group including sham group) were killed by decapitation; the fresh tissues including hippocampus were dissected, frozen in dry ice and stored at −70°C until use for biochemical analyses. Another set of rats was maintained on a regular diet (0.9% DHA and 1% eicosapentaenoic acid, EPA) or special diet containing 8% fish oil (12.4% DHA and 13.5% EPA) for 4 weeks before a mild FPI or sham surgery was performed. Rats were killed at day 7 after surgery. In regular diet: total fat content, 4.5%; total saturated fat, 1.5%; total monounsaturated, 1.58%). In the 8% fish oil diet: total fat content: 10.4%; total saturated fat, 2.8%; total monounsaturated, 2.29%). The diets, fed *ad libitum*, were provided in powder (TestDiet Inc., Richmond, IN) in large bowl and contained a standard vitamin and mineral mix with all essential nutrients. This set of rats were divided into four groups: Sham-RD (regular diet), TBI-RD, Sham-FO (fish oil), and TBI-FO.

All experiments were performed in accordance with the U.S. National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and animal suffering was minimized.

Fluid Percussion Injury

The injury was performed as previously described (Wu et al., 2003). In brief, with the aid of a microscope (Wild, Heerburg, Switzerland) a 3.0-mm-diameter craniotomy was made 3.0 mm posterior to bregma and 6.0 mm lateral (left) to the midline with a high-speed drill (Dremel, Racine, WI). A plastic injury cap was placed over the craniotomy with silicone adhesive and dental cement. When the dental cement hardened, the cap was filled with 0.9% saline solution. Anesthesia was discontinued and the injury cap was attached to the fluid percussion device. At the first sign of hind-limb withdrawal to a paw pinch, a mild fluid percussion pulse (1.5 atm) was administered. Sham animals underwent an identical preparation with the exception of the lesion. Immediately upon responding to a paw pinch, anesthesia was restored and the skull was sutured. Neomycin was applied on the suture and the rats were placed in a heated recovery chamber for approximately an hour before being returned to their cages.

Western Blot

Hippocampal tissue from surgery side was homogenized in a lysis buffer containing 137 mM NaCl, 20 mM

Tris–Cl pH 8.0, 1% NP40, 10% glycerol, 1 mM PMSF, 10 μg/mL aprotinin, 0.1 mM benzethonium chloride, 0.5 mM sodium vanadate. The homogenates were then centrifuged, the supernatants were collected and total protein concentration was determined according to MicroBCA procedure (Pierce, Rockford, IL), using bovine serum albumin as standard. Sir2α levels were analyzed by western blot. Briefly, protein samples were separated by electrophoresis on an 8% polyacrylamide gel and electrotransferred to a nitrocellulose membrane. Non-specific binding sites were blocked in TBS, overnight at 4°C, with 2% BSA and 0.1% Tween-20. Membranes were rinsed for 10 min in buffer (0.1% Tween-20 in TBS) and then incubated with anti-actin or anti-uMtCK (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA), followed by anti-goat IgG horseradish peroxidase–conjugate (Santa Cruz Biotechnology); anti-Sir2α (1:1000; Upstate, Chicago, IL), anti-AMPK, anti-p-AMPK (Cell Signaling, Danvers, MA) followed by anti-rabbit IgG horseradish peroxidase-conjugate (Santa Cruz Biotechnology). After rinsing with buffer, the immunocomplexes were visualized by chemiluminescence using the ECL kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ) according to the manufacturer's instructions. The film signals were digitally scanned and then quantified using NIH Image software. Actin was used as an internal control for western blot such that data were standardized according to actin values.

Immunohistochemistry

Serial coronal sections (25 μm) were cut on a cryostat, mounted to gelatin-coated slides and processed for immunohistochemistry, as previously described (Gomez-Pinilla et al., 2001). A 1:1000 dilution was used for the rabbit polyclonal anti-Sir2 (Chemicon International Inc., Temecula, CA). Immunohistochemistry controls were performed by omission of the primary antibody. The results of immunohistochemistry controls were negative as no staining was observed in cell structures.

Measurement of Oxidized Proteins

The amounts of oxidized proteins containing carbonyl groups were measured by using an Oxyblot kit (Intergen, Purchase, NY). Briefly, the protein sample (10 μg) was reacted with 1× dinitrophenylhydrazine (DNPH) for 15 min, followed by neutralization with a solution containing glycerol and β-mercaptoethanol. These samples were electrophoresed on an 8% polyacrylamide gel and electrotransferred to a nitrocellulose membrane. After blocking, membranes were incubated overnight with a rabbit anti-DNPH antibody (1:150) at 4°C, followed by incubation in goat anti-rabbit (1:300) for 1 h at room temperature. After rinsing with buffer, the immunocomplexes were visualized by chemiluminescence using the ECL kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ) according to the manufacturer's instructions.

Statistical Analysis

Actin was employed as internal standard for western blot. For Western blot, the values were expressed as a ratio of actin value and then converted to percent of control as presented in bar figures and represented the mean ± SEM. The data were analyzed by ANOVA followed by Fisher's protected least significance *post hoc* test. Statistical differences were considered significant at $p < 0.05$.

RESULTS

Sir2α Expression

Previous studies indicated that TBI results in cumulative reactive oxygen species associated with impairment in cognitive abilities (Wu et al., 2004). Given the potential role of Sir2α in the anti-oxidant protection (Daitoku et al., 2004), we determined whether Sir2α expression might be affected by TBI. Our results demonstrated that Sir2α level was significantly decreased in TBI rats at day 7 (75% of sham control), and day 14 (82% of sham control) after lesion ($p < 0.05$; Fig. 1A) than that in sham animals. No change of Sir2 was found at day 1 after TBI.

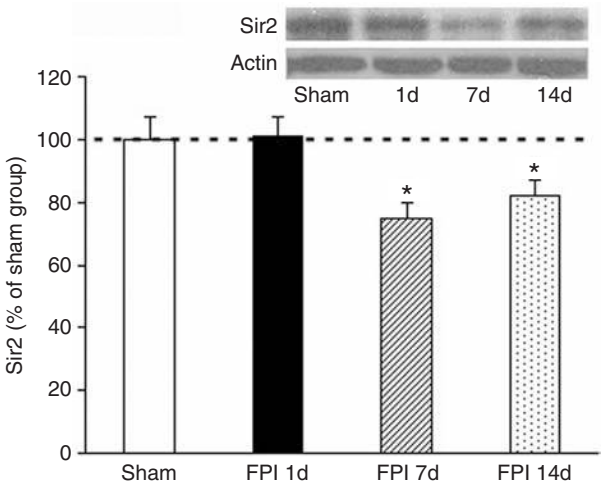

FIG. 1. Expression of Sir2 in rat hippocampus at three time points after FPI revealed by Western blot analysis. Sir2 expression was significantly reduced at day 7 and 14 after FPI. The values were converted to percent of RD group (mean ± SEM). *$p < 0.05$.

It has been well known that omega-3 fatty acids are critical factors for membrane fluidity and integrity. To determine whether omega-3 fatty acids supplementation might restore the level of Sir2α in the hippocampus after TBI, we performed this experiment by feeding a subgroup of animals with diet containing 8% fish oil. Our results indicated that Sir2α was significantly reduced after TBI, whereas fish oil supplementation significantly increased the levels of Sir2 in TBI rats (Fig. 2).

Immunohistochemistry showed that Sir2α staining was predominantly distributed in the mossy fiber system, and in the granule layer of the DG in the hippocampal formation (Fig. 3). Rats showed a qualitative reduction in Sir2α immunoreactivity in the hippocampus after FPI, whereas omega-3 fatty acids supplementation counteracted this reduction (Fig. 3).

Oxidized Protein Levels

Oxidative damage was assessed by using Western blot analysis of DNPH-derivatized carbonyl groups on oxidized proteins as shown in Figure 4. TBI resulted in increased levels of oxidized protein (253% of sham control) compared to sham animals, whereas fish oil supplementation dramatically reduced the oxidized protein levels (30% of sham control; Fig. 4 A,B). In addition, we found that there was a negative correlation between oxidized protein levels and Sir2 levels in TBI rats (RD and FO; $p = -0.67$, $p < 0.05$; Fig. 4C); however, the correlation was greater for the TBI-RD group ($p = -0.81$, $p < 0.05$; Fig. 4C).

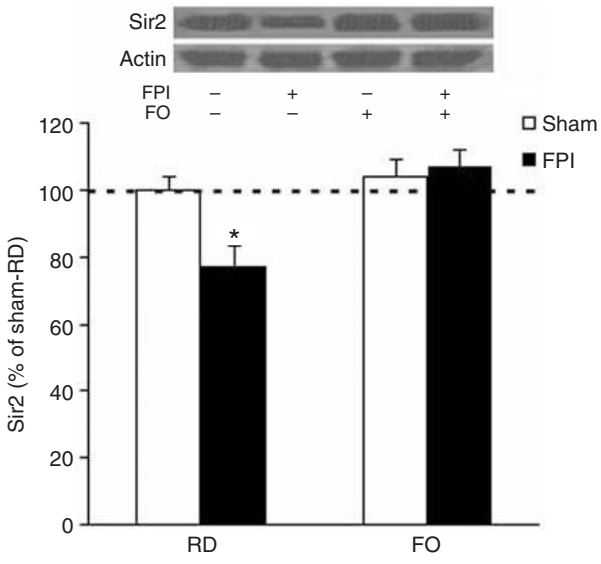

FIG. 2. Expression of Sir2 in rat hippocampus revealed by Western blot analysis. Sir2 expression was significantly reduced after FPI, whereas fish oil supplementation significantly increased the levels of Sir2 in TBI rats. The values were converted to percent of RD group (mean ± SEM). *$p < 0.05$. FO, fish oil; FPI, fluid percussion injury.

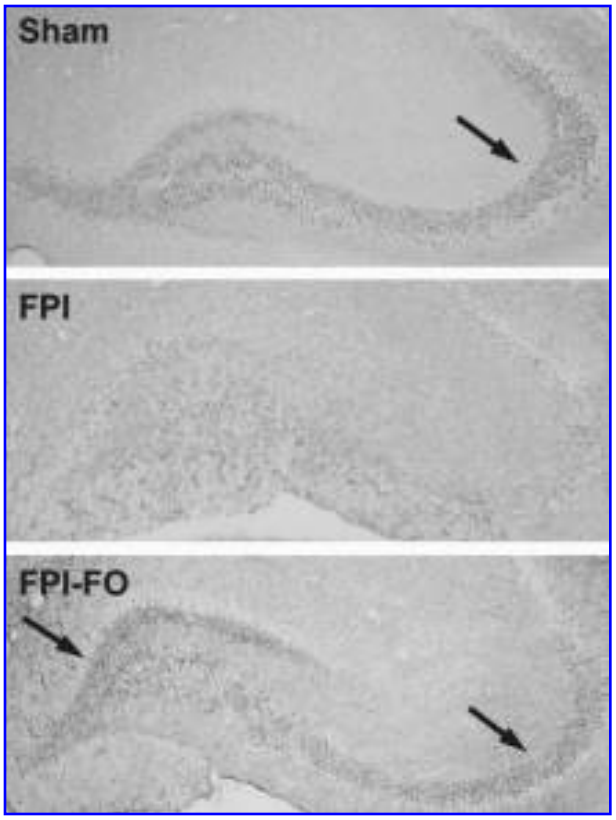

FIG. 3. Sir2 distribution in the hippocampus revealed by immunohistochemistry. Sir2 staining was predominantly distributed in the mossy fiber system between CA3 and the dentate gyrus (DG), and in the molecular layer of the DG of the hippocampal formation. TBI resulted in reduced Sir2 staining in CA3 and DG, but fish oil can counteract the effect of TBI, preserving the Sir2 expression in these subregions of hippocampus. Arrows indicate strong immunostaining for Sir2. RD, regular diet; FO, fish oil.

AMPK and p-AMPK Levels

We have evaluated the role of AMPK in our paradigm given the association of Sir2α with energy metabolism and the newly discovered roles of AMPK in maintaining energy balance (Gadalla et al., 2004; Rafaeloff-Phail et al., 2004; Shaw et al., 2004; Lee et al., 2005). We measured levels of total AMPK and phosphorylated AMPK using western blot analysis in the same tissue used for Sir2α measurements. Our results showed that AMPK and p-AMPK levels were significantly reduced in the hippocampus of rats after FPI compared to sham animals fed

regular diet (80% of sham control for AMPK, 83% of sham control for p-AMPK, $p < 0.05$; Fig. 5A,C). Omega-3 fatty acids supplementation normalized levels of AMPK and p-AMPK in FPI rats (96% of sham control for AMPK and 95% of sham control for p-AMPK in TBI rats fed fish oil; $p > 0.05$; Fig. 5A,B).

We performed a correlation analysis between Sir2 and AMPK to determine whether the change of Sir2 is associated with the alteration of AMPK. We found that Sir2 levels were positively correlated with AMPK levels in sham rats fed regular diet ($r = 0.82$, $p < 0.05$). This correlation was disrupted by FPI ($r = -0.46$, $p > 0.05$; Fig. 5B), which was restored by fish oil supplements ($r = 0.88$, $p < 0.05$). Similar results were found for the correlation between Sir2 and p-AMPK. The positive correlation ($r = 0.96$, $p < 0.05$) in sham animals fed regular diet was disrupted by FPI ($r = -0.42$, $p > 0.05$), which was reversed by fish oil supplementation ($r = 0.88$, $p < 0.05$; Fig. 5D).

uMtCK Levels

We found that FPI reduced ubiquitous mitochondrial creatine kinase (uMtCK, 80% of sham, $p < 0.05$; Fig. 6),

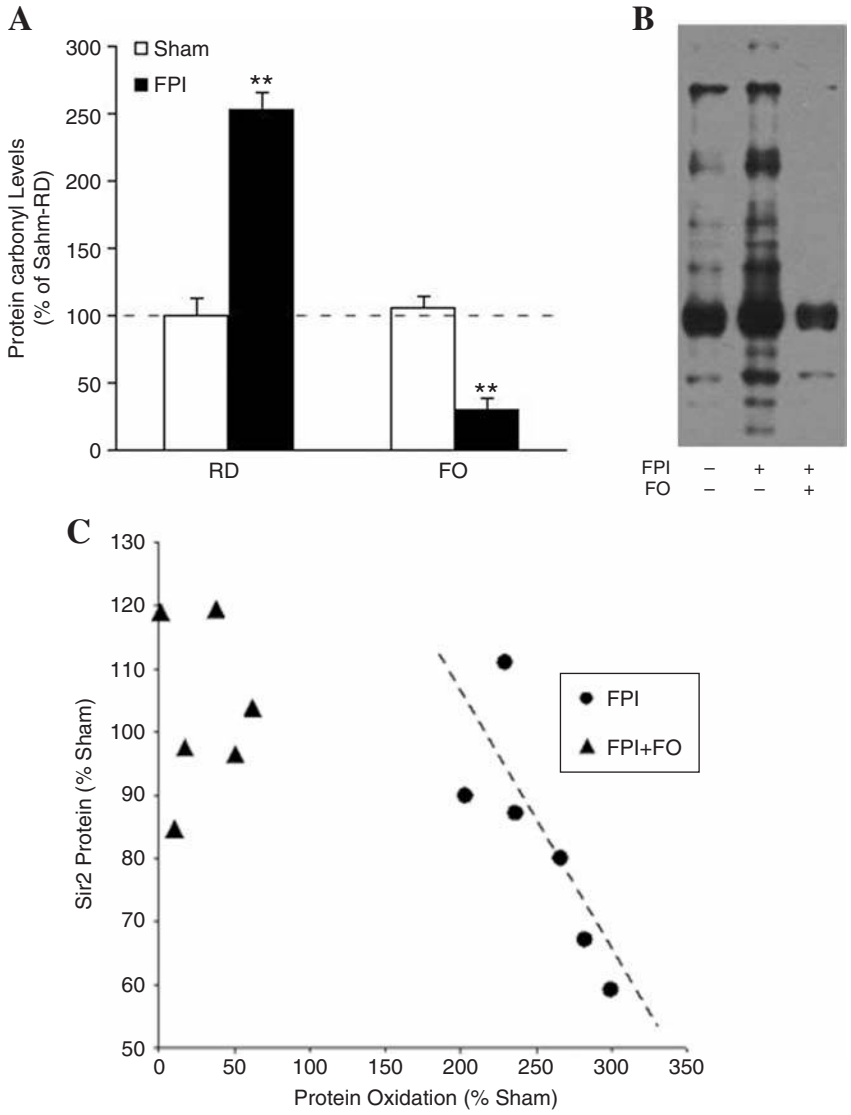

FIG. 4. Measurement of oxidized protein levels in rat hippocampus by Western blot analysis of DNPH-derivatized carbonyl groups on oxidized proteins. Note that the oxidized protein levels were significantly increased by TBI, but reduced by fish oil supplement (**A**,**B**). $^{**}p < 0.01$. FO, fish oil; FPI, fluid percussion injury. (**C**) There was a negative correlation between oxidized protein levels and Sir2 levels in TBI rats (RD and FO); however, the correlation was greater for the TBI-RD group (C).

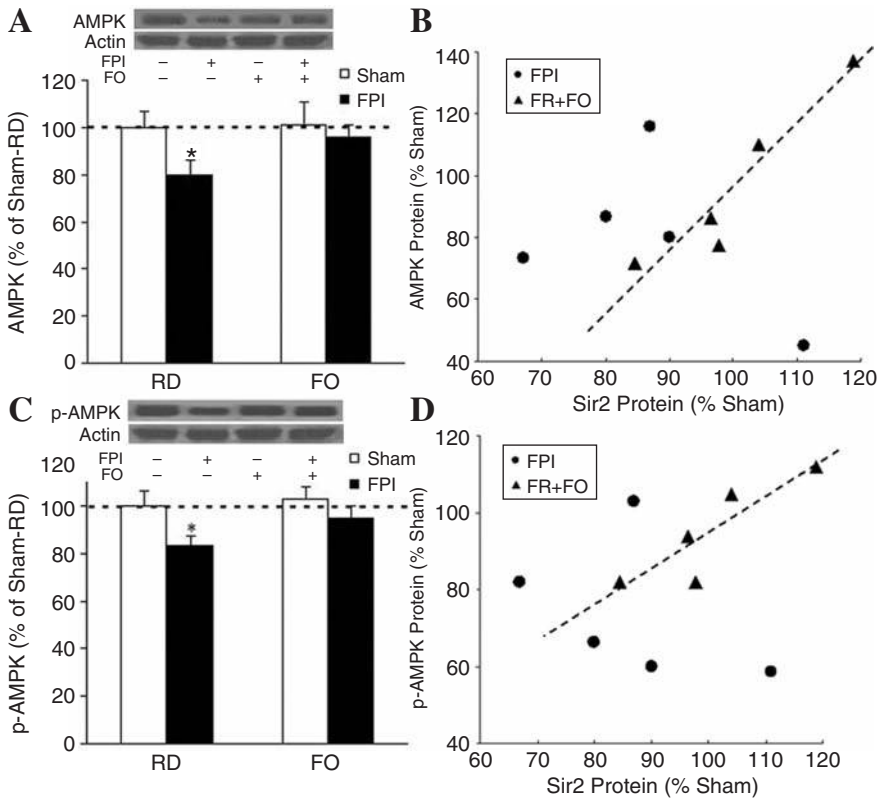

FIG. 5. AMPK and p-AMPK protein levels in rat hippocampus detected by Western blot analysis. TBI resulted in decreased protein levels of AMPK and p-AMPK, whereas fish oil supplementation increased the levels of AMPK and p-AMPK in TBI rats (**A**,**C**). The values were converted to percent of RD group (mean ± SEM). *$p < 0.05$. FO, fish oil; FPI, fluid percussion injury. There was a positive correlation between Sir2 and AMPK in sham rats. This correlation was disrupted by TBI, but restored by fish oil supplement (**B**). The similar results were found for the correlation between Sir2 and p-AMPK (**D**).

an important enzyme implicated in the energetic regulation of Ca^{2+}-pumps and in the maintenance of Ca^{2+}-homeostasis, whereas omega-3 fatty acids supplements increased the levels of uMtCK after lesion (126% of sham control; Fig. 6).

DISCUSSION

Sir 2, originally described for its involvement in longevity in yeast, is starting to be evaluated for its putative capacity to provide neuronal protection under challenging conditions (Parker et al., 2005; Sinclair, 2005; Wu et al., 2006). Our current findings indicate that TBI reduces the expression of Sir-2, in conjunction with levels of energy metabolic markers in the hippocampus. Dietary supplementation of omega-3 fatty acids counteracted the effects of TBI on reducing the levels of Sir2 and energy metabolic markers. Our results indicate a possible role of oxidative metabolism on mediating the effects of TBI on neuronal plasticity using Sir2 as a main mediator. These results showing that omega-3 fatty acids supplementation normalizes levels of Sir2 may uncover one of the underlying mechanisms by which this diet protects the brain against insults (Wu et al., 2004).

Omega-3 Fatty Acid Diet Counteracts the Decrease in Sir2 after TBI

Our results showed that dietary supplementation of omega-3 fatty acids reverses the decrease in Sir2α in the hippocampus after TBI, proportionally to a decrease in levels of oxidative stress. Previous studies have shown that dietary supplementation of omega-3 fatty acids has a strong antioxidant capacity following TBI (Wu et al., 2004). The combined evidence seems to indicate that the ability of omega 3 fatty acids to normalize Sir2α levels after trauma is achieved by reducing oxidative stress. Sir2α has been previously implicated in the cellular homeostatic mechanisms that modulate oxidative stress (Araki et al., 2004; Brunet et al., 2004; Cohen et al., 2004; Daitoku et al., 2004; Wu et al., 2006). For example, Sir2 can increase the ex-

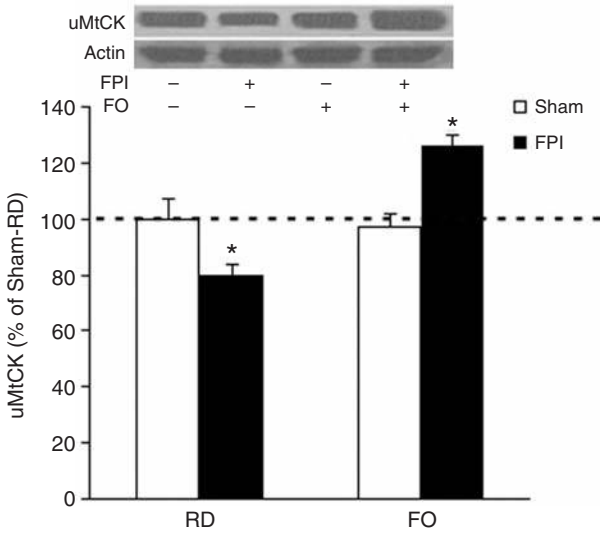

FIG. 6. uMtCK protein levels in rat hippocampus detected by Western blot analysis. TBI resulted in decreased protein levels of uMtCK, whereas fish oil supplementation increased the levels of uMtCK in TBI rats. The values were converted to percent of RD group (mean ± SEM). *$p < 0.05$. FO, fish oil; FPI, fluid percussion injury.

pression of manganese superoxide dismutase (MnSOD), a potent anti-oxidant enzyme, which in turn can increase the cellular aptitude to detoxify reactive oxygen species (Kops et al., 2002; Nemoto and Finkel, 2002; Brunet et al., 2004; Daitoku et al., 2004). Resveratrol, a powerful antioxidant molecule found in food (i.e., grapes, peanuts, and red wine) activate Sir2α (Parker et al., 2005; Anekonda and Reddy, 2006). Elevated levels of Sir2α promoted by the antioxidant resveratrol have been shown to suppress the neuronal death caused by genetic overexpression of huntingtin, whereas the Sir2α inhibitors sirtinol and nicotinamide blocked the action of resveratrol (Parker et al., 2005; Sinclair, 2005). Oxidative stress is a byproduct of dysfunctional energy metabolism, a prevalent condition after TBI. The overall results suggest that oxidative metabolism can be a pivotal mechanism for maintaining normal levels of Sir2 in the brain, and that levels of Sir2 can be manipulated by dietary factors. Figure 7 illustrates the possible mechanisms and rationale for Sir2 up-regulation and action. In this study, we found that FPI reduced levels of Sir-2 in the hippocampus, and that this reduction was proportional to an elevation in protein oxidation. Accordingly, we found a significant negative correlation between oxidized protein levels and Sir2 levels in TBI rats (RD and FO); however, the correlation was greater for the TBI rats fed regular diet. Consistently, the oxidized protein levels were much higher in TBI rats compared to sham animals. The fact that elevated oxidative stress is a prevalent condition in the traumatically injured brain; our results seem to indicate that oxidative stress may be a factor for decreased Sir2 after TBI. We found that omega-3 fatty acid diets did not affect Sir2 levels in the hippocampus in sham animals, but reversed the reduced Sir2 levels after TBI. It has been shown that elevated oxidative stress may reduce the Sir2 levels (Wu et al., 2006). Thus, the omega-3 fatty acids may combat the TBI-induced reduction of Sir2 through reducing the oxidative stress. However, further investigations are needed to explore underlying mechanisms.

We cannot discard the possibility that the omega-3 fatty acids diet may promote neuronal viability in conditions of elevated neuronal death. The fact that the mild FPI used in our study involves minimal cell death precludes measuring the effects of omega-3 fatty acids on promoting cell viability. The fact that levels of Sir2 were unchanged in sham rats treated with omega-3 fatty acids, is in line with evidence supporting a role of Sir2 predominantly under challenging conditions.

Sir2α Expression and Energy Metabolism after TBI

To further explore a possible modulatory role of oxidative metabolism on Sir2, we measured AMPK and

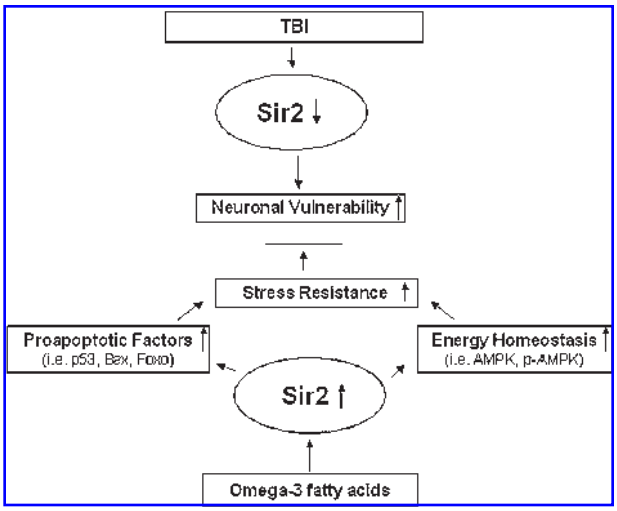

FIG. 7. Possible mechanisms underlying the beneficial effects of omega-3 fatty acids dietary supplementation on the injured brain. Sir2 may serve general mechanisms that provide protection to the brain against insults; therefore, a reduction of Sir2 as a consequence of brain injury may increase neuronal vulnerability to insults. In turn, dietary supplementation of omega-3 fatty acids may protect the brain from the burden of trauma by maintaining levels of Sir2. Sir2 may provide neuroprotection by using mechanisms that promote stress resistance such as induction of antioxidant gene expression and maintaining energy homeostasis.

uMtCK in animals exposed to TBI and the fish oil diet. TBI reduced the levels of AMPK and p-AMPK in our study, and these results are consistent with abundant evidence showing that FPI elicits a metabolic depression in the injured tissue that may last for longer than a week. In addition, our results showed that dietary supplementation of omega-3 fatty acids restored levels of total AMPK and p-AMPK, in proportion to the levels of Sir2α. The apparent association between Sir2 and AMPK suggest an involvement of energy metabolism on Sir2 modulation. Accordingly, we found a strong association between Sir2 and AMPK and its activated state, which was disrupted by TBI. The findings that omega-3 fatty acids supplementation reversed the reduced levels of Sir2, AMPK, p-AMPK, and restored the association between Sir2 and AMPK systems after lesion, implicate oxidative stress in the adverse effects of TBI on the energy homeostasis in which Sir2 is involved.

The reversal of metabolic changes by omega-3 fatty acids supplemented diets may help improve the outcome of neurological disorders. For example, stimulation of cerebral AMPK, a major intracellular energy sensor, can ameliorate cerebral dysfunction following liver failure (Dagon et al., 2007). Another example is that a balanced interaction between angiogenic IGF-I and AMPK signaling may be necessary for proper vascular homeostasis that, in turn, improves cognitive performance in Alzheimer's disease (Lopez-Lopez C et al., 2007). We have previously shown that the uncoupling protein 2 (UCP2) may play a crucial role interfacing energy homeostasis and synaptic plasticity. Further investigations are needed to explore the possibility that the omega-3 fatty acid diet may increase neuroprotection by acting on UCP2.

We further found that TBI reduced the levels of another important marker of cellular energy such as ubiquitous mitochondrial creatine kinase (uMtCK). Our results suggest that increased oxidative stress may be associated with the decreased uMtCK by TBI. We found that omega-3 fatty acids supplementation normalized levels of uMtCK, a condition that may be related to the antioxidant actions of this dietary supplementation. uMtCK is an important enzyme implicated in the energetic regulation of Ca^{2+}-homeostasis. uMtCK is important for mitochondrial energy channeling (Wallimann et al., 1992 and Wallimann et al., 1998) and permeability transition (O'Gorman et al., 1997 and Dolder et al., 2003), and has been shown to protect against oxidative or toxic insults (O'Gorman et al., 1997, Kanazawa et al., 1998, Dolder et al., 2003 and Hatano et al., 2004). It has been reported that genetic deletion of uMtCK leads to impaired learning and memory in mice (Streijger et al., 2004), suggesting that uMtCK reduction may play an important role in cognitive deficits seen in TBI. Therefore, the deceased level of uMtCK after FPI may provide important insights to better understand how TBI can affect neural function by compromising energy metabolism and associated molecular systems such as Sir2.

How Sir2α May Benefit the Injured Brain?

Abundant evidence indicates that the acute phase of TBI is characterized by a reduction in metabolic energy that can affect neuronal survival and function (Tanaka et al., 2005). Accordingly systems that can restore energy are likely beneficial for neuronal function after TBI. Recent studies have provided evidence that Sir2 facilitate molecular mechanisms that facilitate the transference of energy to cells. Sir2α has been implicated in the regulation of transcription factors including FOXO and PGC-1α that control energy homeostasis. It has been show that in liver cells, Sir2α can coordinate with PGC-1α to activate gluconeogenic gene expression in response to fasting. Further, a new study suggests that Sir2 controls mammalian Acetyl-CoA synthetase 1 (AceCS1). Importantly, the AceCS is the only mammalian path to convert free acetate back to a useable metabolite such as acetyl-CoA. It is known that AceCS catalyzes the formation of acetyl-CoA from acetate, CoA and ATP. Accordingly, the overall evidence seems to indicate that normal levels of Sir2 may be important to maintain cellular homeostasis required for neural plasticity following TBI.

It is noteworthy that Sir2 has a recognized role as a histone deacetylator with the capacity to modulate the functional expression of genes important for brain plasticity and function. Accordingly, our results showing that TBI and dietary factors affect the hippocampal expression of Sir2 suggest that Sir2 may be an important mediator for the action of challenges and lifestyle factors on brain plasticity.

Implications of Sir2α Up-Regulation by Fish Oil

Our results provide novel evidence that TBI impacts Sir2α expression in the mammalian hippocampus, a brain region implicated in synaptic plasticity and learning and memory. It is known that Sir2α can increase the cellular ability to detoxify reactive oxygen species (Kops et al., 2002; Nemoto and Finkel, 2002; Brunet et al., 2004; Daitoku et al., 2004). In addition, Sir2α, on its capacity of NAD-dependent histone deacetylase can increase the cellular resistance to cellular stress (i.e., inducing expression MnSOD, subsequently increasing the cellular abilkity to reduce ROS) (Brunet et al., 2004; Daitoku et al., 2004). Our findings suggest that oxidative stress, as an integral consequence of dysfunctional energy homeostasis, is crucial for modulating Sir2 levels. It is signif-

icant that the TBI-induced reduction in levels of Sir2 can be counteracted by dietary supplementation of omega-3 fatty acids, with important implications for regulation of short- and long-term neuronal plasticity. In the context of our results, it is possible to argue that a reduction of Sir2α may confer vulnerability to neurons to secondary damage ultimately affecting the capacity of the brain to cope with insults. For example, TBI is considered as a risk factor for acquiring Alzheimer's disease and other degenerative diseases, and it would be highly relevant to have a dietary strategy for people at a high risk of brain injury such as boxers or brain surgery patients. Therefore, it is critical to increase the understanding of specific mechanisms responsible for long-term plasticity. The results of the present study showing the impact of omega-3 fatty acids dietary supplementation on molecular substrates of plasticity and homeostasis in the injured brain suggest a potential value of this diet to protect the brain against insults.

ACKNOWLEDGMENTS

This study was supported by NIH awards (NS 48804 and 50465) and UCLA Brain Injury Research Center.

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