The role of regular physical activity on proteasome complex in traumatic brain injury

Outlined Booklet of the PhD Thesis

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I. Introduction

Regular exercise is known to improve the physiological performance of skeletal and cardiac muscle and decrease the incidence of a wide range of diseases, including heart and vascular diseases, certain kind of cancer, diabetes II, etc. In the last decade, it became evident that regular exercise beneficially affects brain function, as well as having the potential to play an important preventive and therapeutic role in stroke, Alzheimer and Parkinson diseases. However, this point can be viewed differently, namely that physical inactivity has very serious consequences on the body, including the structure and function of the brain. The effects of exercise and physical activity appear to be very complex and may include neurogenesis via neurotrophic factors, increased capillarization, decreased oxidative damage, and increased proteolytic degradation by proteasome and neprilysin .However, the effect of exercise on brain damage recovery and prevention is studied extensively; although the underlying mechanisms are not well known. One of these mechanisms-which can be key factor for keeping brain healthy-the effects of proteasomes. They are responsible for the degradation of most cytosolic proteins and specific regulatory proteins such as transcription factors associated proteins and cell regulators. While the proteasomes are involved in the degradation on cytoplasmic proteins in general, they have a prominent role in eliminating altered

proteins such as oxidatively damaged, misfolded, or unassembled proteins that are potentially harmful to cells. A human study applying eccentric muscle action causing muscle damage revealed that eccentric exercise caused a decrease in calpain 3 mRNA immediately after the exercise, whereas the calpain 2 mRNA level increased at the day 1. In contrast, cathepsin B+L and proteasome enzyme activities were increased at the day 14 (Feasson et al. 2002). These findings nicely demonstrate that proteolytic processes occur in a selective manner and play an important role in muscle remodelling after injury. The role of proteasome complex in brain remodelling after injury is still unknown.

The first (age-related) study outlined the relationship between the accumulation of oxidative damage to proteins, reactive carbonyl derivative (RCD), and certain brain functions. A spin trapping agent of PBN was administered for two weeks to aged and young gerbils. Following this period, the activities of glutamine synthase and proteasome increased while the level of RCD decreased significantly. These changes were accompanied by improved brain function, as measured by the Morris maze test. Although the findings of this study were questioned at the time.

II. Aims

Hypothesis

1. Based on the available information, we proposed that the effects of exercise on cognitive function and neurotrophins would be reversible.
2. It was proposed that exercise attenuates the level of oxidative stress, and detraining results in enhanced degree of protein and DNA damage in the brain.
3. We hypothesized that the activity of 8-oxoG repair enzyme, OGG1 is induced by exercise.
4. We suggested that exercise attenuates the damage caused by traumatic brain injury.
5. Since traumatic brain injury results is oxidative damage, we hypothesized that proteasome complex plays an important role in the recovery process.
6. It was suggested that early gene expression of Zif268 changed in concert to proteasome in FPI model.

III. Methods

Experiment I

Twenty one male Wistar rats (13 month old) were used in the study Seven rats were randomly assigned to each of the three groups: control (C), exercise trained (ET) and detrained (DT) group. ET and DT rats were subjected to swimming exercise for 8 weeks. After the 8 week exercise training the DT group was kept as the control group for another 8 weeks. Passive avoidance test was used to assess the memory.

Biochemical assays

The concentrations of BDNF and NGF were determined, from the hippocampal section of the brain.

Proteasome activity and content

The chymotrypsin-like activity of proteasome complex from nucleus was determined fluorometrically.

Electron paramagnetic resonance

The electron paramagnetic resonance (EPR) measurements were carried out to determine the ROS in the cerebellar region.

Experiment II.

A total of 33 male Sprague–Dawley adult rats (250–300 g) were utilized in these experiments. Rats underwent lateral fluid percussion injury (FPI; *n*=14) or sham injury (*n*=16) and were housed with or without access to a running wheel from post injury day 0 to 14. All animals were continually monitored and cared for by an IACUC-approved veterinary care staff upon arrival at UCLA. Rats were individually caged with or without access to a running wheel (RW) from post injury day 0 to 14 [Sham-RW (*n*=8) or FPI-RW (*n*=8)]. This post injury period was selected given that it has previously been associated with behavioural deficits. Exercising animals were placed in standard cages equipped with running wheels (diameter=31.8 cm, WIDTH=10 cm; Nalge Nunc International) that rotated against a resistance of 100 g. Wheel revolutions were recorded using an appropriate software (VitalViewer Data Acquisition System; Mini Mitter, Sunriver, OR). Sedentary animals (Sed) were left undisturbed in their home cages [Sham-Sed (*n*=8) or FPI-Sed (*n*=6)].

Fluid percussion injury

Lateral fluid percussion injury has been done accordingly to the method described previously.

Proteasome Activity measurement

The chymotripsin-like activity of proteasome was measured from caudal cortex. The brain was homogenized (100mg) in a 10x volume lysis buffer containing 10mM Tris, 0.25M Sucrose, 1.5 mM MgCl2, 1 mM DTT, 10% Glycerol, 10 KCl, 5mM ATP, complete solution should be around 7.3-7.5. The homogenates were centrifuged, the supernatants were collected and total protein concentration was determined by Micro BCA procedure (Pierce, Rockford, IL) using bovine serum albumin as a standard. The samples were diluted to same concentration (1mg/ml). For the assay, we mixed 5x reaction buffer (500mM Tris-HCl pH 8.0, 5 mM DTT, 25 mM MgCl2), homogenization buffer, BSA, SDS, sample, and substrate (Suc-leu-Leu-Val-Tyr-AMC) to measure cymotripsin like activity of proteasome complex. Incubated the samples at 37 C for 30 min, blocked the reaction with ice-cold methanol, centrifuged the samples at 10,000 g for 5 min we took the supernatant and mixed with Tris-HCl buffer (pH 9.0) and we read the fluorescent intensity at Ex: 320nm Em: 460nm.

Protein Measurements by western blotting

Synapsin I, Zif 268, 20S alfa proteins were analyzed by Western blot as previously described (Griesbach et al.2004). Membranes were incubated with the following primary antibodies: anti-synapsin I (1:2000; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), anti-total Zif 268 (1:2000; Cell Signalling Technology, Inc., Beverly, MA, USA), anti-20 alfa subunit of proteasome (1:2000; Cell Signalling Technology, Inc.), Biotechnology), and anti-actin (1:2000; Santa Cruz Biotechnology) followed by anti-goat IgG horseradish peroxidase conjugate for synapsin I, and actin.

Statistical analysis

At the experiment 1.

The statistical significance was assessed by ANOVA, followed by Tukey's posthoc test and Pearson's correlation. The significance level was set at $p < 0.05$.

At the experiment 2.

An analysis of variance (ANOVA) with repeated measures was used. A Fischer-test was used for cross group comparisons. Results were

expressed as the mean percent of control values for graphic clarity and represent the mean ± standard error of the mean (SEM).

IV.Results

Experiment I

The brain performance, assessed by passive avoidance test, was improved significantly by exercise training, but after de-training, the control and detrained animals showed similar results.

On the other hand, the level of free radical species in the cerebellum decreased as a result of exercise training and this beneficial effect was not eliminated by detraining.

Since, the activity of DNA repair enzyme, OGG1, did not change either in nucleus or in mitochondria by training or detraining, lower level of free radicals production and unchanged OGG1 activity suggest better protection of nuclear and mitochondrial DNA.

The proteasome activity and content were measurable in nucleus, but none of them was altered by training or detraining.

Finally, training significantly increased the protein content of BDNF compared to control animals, while detraining resulted in significantly decreased level of BDNF compared to sedentary rats

Experiment II.

Our data suggest that FPI induced oxidative stress, since the carbonyl concentration increased significantly in caudal cortex of these animals compared to control. On the other hand voluntary exercise decreased the accumulation of carbonyl groups in both sham and FPI groups, suggesting either an increased resistance or increased repair of oxidative protein damage.

Synapsin I is one of the well accepted marker of brain plasticity. Here we evaluated whether FPI and/or exercise changes the protein concentration of synapsin. Our data revealed that as a result of FPI the content of synapsin decreased significantly, on the other hand exercise induced the protein level of synapsin. Those rats which exercised showed no significantly altered synapsin levels from control rats, suggesting beneficial effect of voluntary exercise on FPI via synapsin I.

Based on this suggestion, we have checked the correlation between oxidative protein damage, assessed by level of carbonyl groups in amino acid residues and synapsin I protein concentration. Our data shows that strong negative relationship, which is just in accordance to the belief that oxidative stress results in oxidative damage, which further cause decreased brain plasticity.

The further get information on the possible reason of increased level of carbonyl group accumulation, we tested the activity of proteasome, which is a powerful housekeeping enzyme, which leads to degradation of oxidized proteins. We found, that the activity of proteasome was reduced by exercise and FPI attenuated this decrease.

The correlation between carbonyl group concentration and proteasome activity showed positive relationship, suggesting that lower level of oxidative stress, accumulation of carbonyl groups, is associated with decreased activity of proteasome enzyme. Lack of damage decreases the activity of houskeeping enzyme.

Not only just the activity but the protein content of 20S subunit of proteasome complex also reduced by the lack of the accumulation of carbonyl groups.

The early gene of Zif 268, which is believed to regulates the content of proteasome. Here we indeed showed, first time according to our knowledge in exercise model, that the protein level of Zf 268 showed very similar changes as the content and activity of proteasome.

V. Discussion

At the beginning of the study based on the available information on the related literature we have stated different hypothesis. After the study, here we show whether or research hypothesizes were supported or opposed by the results of our two investigations.

Based on the available information we suggested that the effects of exercise on cognitive function, neurotrophins are reversible.

This hypothesis was correct, and indeed our results showed, except the concentration of free radicals, that the effects of exercise training, in general, reversible. Here we also have to note that the time frame does completely allowed to study the complex reversibility.

It was proposed that exercise attenuates the level of oxidative stress and detraining results in enhanced degree of protein and DNA damage in the brain.

One of the novel observations of our study was that the level of free radicals decreased as a result of exercise training, measured by ESP, but the level of oxidative damage did not increase, therefore just part of this hypothesis was valid.

We hypothesized that the activity of 8-oxoG repair enzyme, OGG1 is induced by exercise.

This hypothesis was not valid, since neither exercise training nor detraining altered the activity of OGG1.

We suggested that exercise attenuates the damage caused by traumatic brain injury.

This hypothesis was supported by our experimental data, and indeed voluntary exercise had beneficial effect on FPI induced damage.

Since traumatic brain injury results is oxidative damage, we hypothesized that proteasome complex plays an important role in the recovery process.

Here we have shown first time, according to our knowledge, that voluntary exercise effects proteasome activiy, which is involved in the remodeling process of the brain after FPI. This hypothesis was correct.

It was suggested that early gene expression of Zif268 changed in concert to proteasome in FPI model. **Indeed, we have shown, according to our suggestion that the early gene of Zif268 plays in important role. This hypothesis was correct.**

VI. Conclusion

In our current investigations we have confirmed that exercise has beneficial effects on brain function. On the other hand, we have generated some novel information by our studies. We have proved that the effects of exercise on brain are reversible, since the functional parameters were declined by detraining. This is expected, but still very important information, which suggest the importance of lifelong exercise, similarly to the lifelong learning. Our study further revealed the importance of oxidative stress and neurotrophins as potential regulatory factors in cognitive function. Upon our finding, number of relevant questions may be asked about the positive or negative effects of antioxidant supplementation on non-deficient subjects. We further gain new information about the DNA damage repair mechanisms. The quality control of DNA is one of the highest priorities of the living cells, since the accumulation of the oxidative damage readily jeopardizes the fate of the cells. Here we have shown, that neither exercise training nor detraining results in that size of challenge to nuclear or mitochondrial DNA, which could result in induction of OGG1, which is one of the most important enzymes in the base excision repair process of 8-oxoG. Similarly, with this

observation, it is safe to conclude that neither exercise nor detraining was powerful enough to alter the protein content of the mitochondrial electron chain complexes. In our second study, we have reached also some important conclusions. The most important one could be that mimicked traumatic brain injury by fluid percussion injury results in oxidative stress and accumulation of carbonyl groups, which could be significantly prevented by voluntary exercise. In addition, furtherobservation on the involvement of proteasome in remodeling of brain after FPI was gained. Simply put, the effects of exercise on proteasome complex appear to be very important to reduce the oxidative damage caused by FPI and regulate brain plasticity. We also have shown that this process includes synapsin I and Zif 268.

VII. PUBLICATION LIST

VII.I. Publications related to dissertation

1. Radak,Z., Toldy,A., **Szabo,Z.,** Siamilis,S., Nyakas,C., Silye,G., Jakus,J., Goto,S., 2006. The effects of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. Neurochem.Int. 49, 387-392. **IF. 3.0**
2. **Szabo Z**, Ying Z, Radak Z, Gomez-Pinilla F., 2009. Voluntary exercise may engage proteasome function to benefit the brain after trauma. Brain Res. In press. **IF. 2.3**

VII.2. Publications independent from dissertation

1. Radak Z**,** Koltai E, Hart N, **Szabo Z.** The role of reactive oxygen and nitrogen species in skeletal muscle. In. **Muscle Plasticity**. Edited:Magalhaes J, Ascensao A) 37-46 p. Research Signpost Karela. 2009

2. Koltai E, **Szabo Z**, Atalay M, Boldogh I, Naito H, Goto S, Nyaks C, Radak Z. Exercise alters SIRT1, SIRT6, NAD and

NAMPT levels in skeletal muscle of aged rats. Mechanism Aeging Development 2010, 131: 21-28 **IF. 3.9**