Spontaneous Wheel Running Exercise Induces Brain Recovery via Neurotrophin-3 Expression Following Experimental Traumatic Brain Injury in Rats

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Abstract. [Purpose] The aim of the present study was to investigate the expression of neurotrophin-3 (NT-3) after applying spontaneous wheel running exercises (SWR) after experimental traumatic brain injury (TBI). [Subjects and Methods] Thirty male Sprague-Dawley rats were divided into 3 groups; 20 rats were subjected to controlled cortical impact for TBI, and then, animals were randomly collected from the SWR group and subjected to wheel running exercise for 3 weeks. Ten rats were not subjected to any injury or running exercise to compare with the effect of TBI and SWR. Immunohistochemistry, Western blotting, skilled ladder rung walking test, and 2,3,5-triphenyltetrazolium chloride staining analysis for the evaluation of NT-3 expression were used to assess brain damage and recovery. [Results] The TBI-induced decrease in NT-3 expression was recovered by wheel running exercise. Moreover, decreased ischemic volume and progressive neurobehavioral outcome were observed in the SWR group. [Conclusion] Spontaneous running exercise promotes brain recovery and motor function through an increase in expression of NT-3.

Key words: Neurotrophin-3, Traumatic brain injury, Wheel running exercise

INTRODUCTION

The majority of deaths and disabilities from trauma occur with traumatic brain injury (TBI)1. TBI is an insult to the brain that results in impairments of cognitive and physical functioning as well as disturbances in behavioral or emotional functioning2. Previous studies have attempted to establish laboratory models of TBI to assess the damage phase and treatment in a variety of primates and conditions3. Among them, controlled cortical impact (CCI), occasionally referred to as the rigid percussion model, is a model of traumatic brain injury in ferrets, rats, and mice that may potentially prove useful in elucidating the mechanisms underlying neurodegeneration using genetically altered animals4, 5. The CCI model allows for ready manipulation and accurate quantification of biomechanical forces6. Injury results in a considerable hematoma under the injury site that is immediately visible, and the impact induces primary necrosis at the center of the contusion. Secondary cellular loss in sensitive regions is observed post injury7.

Spontaneous exercise may be therapeutic in the management of CNS injury, by reducing the degree of initiatitary damage, limiting the degree of secondary neuronal death, and promoting neural repair and behavioral rehabilitation8. The effects of exercise on genes encoding for neurotrophins and other proteins suggest that exercise could regulate anatomical changes that support brain plasticity. It has been previously demonstrated that exercise increases the number of new neurons in the dentate gyrus9. Epileptic, ischemic, and traumatic insults to the brain induce marked changes in the expression of genes encoding for neurotrophins, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) in cortical and hippocampal neurons9.

The purpose of the present study was to investigate the expression of NT-3, which provides a neuroprotective effect that promotes the maintenance and survival of neurons in rats undertaking spontaneous wheel running exercise after experimental TBI using a CCI model in rats.

SUBJECTS AND METHODS

Thirty male Sprague-Dawley rats weighing between 250 g and 300 g were used and maintained on a 12 h on/12 h off light/dark cycle with ad libitum access to food and water. All the experiments were performed in accordance with protocols approved by the University of Daegu Animal Experiment Committee, based on the NIH Guidelines for the Care and Use of Laboratory Animals (NIH publica-
and described in detail by previous studies[10, 11]. The NRM sue deformation of 3 mm. The injury device was modified
ma. Injury was induced with a weight 3 mm in diameter
from a height of 20 cm. This resulted in an injury with a tis-

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solved in PBS for 20 min at 37 °C and subsequently trans-
chloride (TTC, Sigma-Aldrich, St. Louis, MO, USA) dis-
samples were incubated in 2% 2,3,5-triphenyltetrazolium
brains were extracted, immersed for 10 min in cold PBS,
Clarion (Biomedia, USA).
series of alcohols, soaked in xylene, and cover-slipped with
dery overnight. The sections were then dehydrated through a
onto gelatin/chromium-coated slides, and allowed to air-

When sacrificed, the animals were anesthetized with a
mixture of 2 mL/kg 50% Zoletil and 50% Xylazine hydrochloride and perfused through the heart with 200 mL of
0.9% NaCl solution followed by 4% paraformaldehyde solu-
tion. The brains were removed, maintained in post-fixative
overnight, and then sectioned to a thickness of 30 µL for
immunohistochemistry.

In brief, the sections were washed (3×10 min) in 0.01 M
phosphate-buffered saline solution (PBS; pH 7.2) and incub-
bated for 12 h at room temperature with mouse monoclonal
anti-NT-3 (Chemicon, Temecula, CA, USA). The antibody
diluted to 1:200 with a solution of Triton X-100 and normal donkey
serum. After incubation in secondary antibody, the sections
were rinsed (3×10 min) in PBS, and then incubated for 1 h
incubation in primary antibodies at the dilutions recom-
pended by the suppliers. The membranes were washed,
and the primary antibodies were detected using horseradish
peroxidase-conjugated goat anti-rabbit IgG or goat-anti
mouse IgG. The bands were then visualized via enhanced
chemiluminescence (Amersham Pharmacia Biotech, Piscata-
way, NJ, USA).

The ladder walk device consisted of a 1 m long wooden-
rung walkway with a varying distance of 1.5 cm between
rungs. Rats were habituated to the testing ladder before TBI
surgery. The animals could traverse the walkway freely
without reinforcement. During the third session, the
animals were video-recorded as they traversed the walk-
way three times. Tapes were later analyzed to obtain pre-
operative baseline scores. Each group was tested at week
1 after surgery, and then once per week for the following 2
weeks. In a single testing session, the animals crossed the
walkway three times and received a score for the number of
fore and hind limb feet faults per each 10 steps. Only the
lesion-affected limbs were analyzed, and a foot fault was
characterized as a total miss or slip from the rung, or a mis-
placement of the paw on the rung. The method was modi-
cified as described previously[13].

The results were expressed as the means±standard error
(SE) or deviation (SD). All experiments were analyzed via
analysis of variance, and some experiments were analyzed
via comparisons of the treatment mean with the controls us-
ing the Bonferroni-Dunn test. Differences were considered
statistically significant when p < 0.05. All analyses were
performed using SPSS for Windows (v. 12.0 K, SPSS Inc.,
Chicago, IL, USA).

RESULTS

Skilled ladder rung walking tests were conducted to
evaluate the effects of spontaneous wheel running after
TBI, which is sensitive to alterations of motor function af-
fter sensory motor cortex damage (Table 1). The error ratio
was measured in the forelimbs (A) and hind limbs (B). The
results for both affected limbs in all three groups showed a
significant decrease in the error ratio compared with the be-
fore exercise period as time passed significantly (p<0.05).
Comparison with the NRM group showed that there was the
more increase in the error ratio in the TBI group. Moreover,
the increase tended to be less profound in the SWR group than in the TBI group (p<0.05).

Immunohistochemistry and immunoblotting for NT-3 expression were performed in each group (Fig. 1 and Table 2). It was observed that the TBI-induced reduction in NT-3 expression increased wheel running for 3 weeks. A more profound significant increase in NT-3 expression was noted in the SWR group than in the NRM group (p<0.05).

To confirm the area of the brain injured by TBI, TTC staining was varied out (Table 3). No ischemic areas were detected in the NRM group. In the SWR group, the ischemic area was statistically significant decrease than in the TBI group (p<0.05).

Table 1. The effect of spontaneous wheel running after TBI on behavior recovery

| Group | Error ratio (%) |
|-------|----------------|
|       | Before exercise | 1 week | 2 weeks | 3 weeks |
| TBI   | 54±9.67         | 36±6.70* | 34±9.67* | 31±8.76* |
| SWR   | 56±8.43         | 41±7.38* | 25±8.50* | 19±8.76* |
| NRM   | 20±8.16         | 15±5.27  | 14±5.16  | 8±7.89  |

A

Skilled ladder rung walking test was conducted for the with forelimbs (A) and hind limbs (B). Each example shown is representative of three experiments. The error ratio (%) is the mean ± SD of the values to measure during the test. Statistical analysis was performed by using one-way ANOVA. * p < 0.05 versus before exercise between periods; † p < 0.05 versus 1 week after exercise between periods; ‡ p < 0.05 versus 2 weeks after exercise between periods; § p < 0.05 versus CON between the group at 3 weeks after exercise.

Fig 1. The effect of spontaneous wheel running after TBI on NT-3 expression in immunohistochemistry

To confirm NT-3 expression, immunohistochemistry (A) was conducted in the NRM (a), TBI (b), and SWR (c) groups as described in the Materials and Methods section. Each example shown is representative of three experiments. Scale bar = 200 μm.

Table 2. The effect of spontaneous wheel running after TBI on NT-3 expression in Western blotting analysis

| Group | Relative optical density (% of TBI at 3 weeks after exercise) |
|-------|-------------------------------------------------------------|
|       | NRM        | TBI        | SWR        |
| 3 weeks| 100.00±1.02 | 79.50±3.48* | 120.77±1.87* |

To confirm NT-3 expression, Western blotting analysis was conducted as described in the Materials and Methods section. Each example shown is representative of three experiments. The optical density values denote the mean ± SE of three experiments for each condition determined from densitometry relative to β-actin, respectively. Statistical analysis was performed by using one-way ANOVA. * p < 0.05 versus NRM. § p < 0.05 versus TBI.
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morphological plasticity of neural cells. Moreover, some of
mote survival, division, growth, and the differentiation and
21). Ad-
exercises, induced increases in the levels of neurotrophic
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tion, and diffuse axonal injury according to the features,

sions1, 16). These brain injuries trigger pathological pathways
and auto-protective mechanisms. Pathological pathways
that may potentially harm brain cells include excitotoxicity,
free radical formation, inflammation, and apoptosis, among
others. Auto-protective mechanisms include the formation of
heat shock proteins (HSPs), anti-inflammatory cytokines,
growth factors (GFs), and endogenous antioxidants17).

Among endogenous protective mechanisms, growth
factors are special endogenous signaling proteins that pro-
mote survival, division, growth, and the differentiation and
morphological plasticity of neural cells. Moreover, some of
these growth factors are required for certain trophic or all of
these functions in selected neural populations of the nervous
system18). Growth factors can generally be divided into four
major groups according to their receptor systems and their
downstream signal transduction pathways. Many types of
growth factors are induced very early after brain lesions,
including NGF, BDNF, glial cell line-derived growth factor
(GDNF), basic and acidic fibroblastic growth factors (FGF),
and members of the transforming growth factor super fam-
ily (TGF). Other new members that were identified include
NT-3 and NT-4/517, 18).

NT-3 was detected in the corpus callosum, the hippo-
campus, cortex (layer V), the primary olfactory cortex, the
amygdala, the Purkinje cells of the cerebellum and spinal
cord, and so on19). It has been suggested that this neuro-
trophin may serve a neuroprotective function by playing a
role in the maintenance and survival of neurons after TBI.
Thus, it is important to characterize the spatial and tempo-
ral patterns and levels of NT-3 expression following experi-
mental brain injury20).

In the present study, NT-3 expression was increased in
the SWR group and was reduced after TBI. It was reported
that physical exercise, such as treadmill and wheel running
exercises, induced increases in the levels of neurotrophic
factor, and this fact is consistent with our findings21). Ad-
ditionally, these neurotrophic factors prevent programmed
cell death and promote cell survival in the progression of
brain injury22). These imply that increased NT-3 expression
as a result of wheel running exercise reduced programmed
cell death and tissue ischemic injury and induced brain re-
covery via the regulation of cellular signaling.

The aim of motor training, such as running exercise, is
to diminish functional disability and promote brain recover-
y. This can be achieved by proper intervention for reha-
bilitation, which is useful in stimulating therapy-induced
recovery in brain-injured patients23). Exercise, as a simple
and widely practiced behavior, can enhance the activation
of molecular and cellular cascades and promote brain vas-
cularization, neurogenesis, and functional changes in neu-
ronal structure, as well as resistance to injury24).

In conclusion, spontaneous running exercise promotes
brain recovery and motor function as the result of NT-3 ex-
pression. These facts imply that spontaneous exercise would
be needed for rehabilitation of brain-injured patients when
properly directed by physical therapist properly. Moreover,
it the present study contributes evidence showing that spon-
taneous rehabilitation training is helpful to biological and
functional recovery in brain injured-patients by stimulating
the metabolic activation in the brain.

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Table 3. The effect of spontaneous wheel running after TBI on the brain-
injured area

| Group | Volume of injury (% of total area at 3 weeks after exercise) |
|-------|----------------------------------------------------------|
|       | NRM | TBI | SWR |
| 3 weeks | 0   | 22.2±3.01 | 5.3±0.67* |

To confirm the ischemic volume of the brain-injured area, TTC staining was
conducted in the NRM, TBI, and SWR groups as described in the Materials
and Methods section. The results provide the percentage of injured area in
total brain area pixels and represent the mean ± SE. Each example shown is
representative of three experiments. Statistical analysis was performed by
using the independent t-test. * p < 0.05 versus TBI.
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