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Different Effect by Different Starting Time Point of Exercise of the Nerve Regeneration after the Peripheral Nerve Injury

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Abstract

Starting time point of exercise intervention is a hot issue in the field of exercise therapy. Some researchers insist that the exercise interventions should started from early period of rehabilitation but the others insist that the exercise interventions should started from later period that followed the first step of recovery period. This hot issue spread to peripheral nerve injury patients and therapist too. After the peripheral nerve injury, injured nerve fibers degenerated from distal to lesion. This degenerating process is known as the Wallerian degeneration.During this Wallerian degeneration process, macrophages remove the debris of axon and myelin that were damaged from injury. Without this cleaning process by macrophages, axons could not reconnect well and the whole process of nerve regeneration can't be perfect. So the recruitment of macrophages during this Wallerian degeneration process could be crucial to peripheral nerve regeneration whole process.

Treadmill exercise is widely used for motor rehabilitation and general health control in many field of therapy. Especially for the peripheral nerve rehabilitation, treadmill exercise interventions used open. So we investigate the effects of treadmill exercise on Wallerian degeneration process and analyze the benefits of treadmill exercise on peripheral nerve regeneration. We had investigated the type of macrophages and biomarkers to analyze the beneficial effects of treadmill exercise intervention on the Wallerian degeneration models.

The main goal of this experiment was to compare and analyze the effects of early started exercise group and later started exercise group. With our study results, we have conclude that the later started exercise had better beneficial effect to the peripheral nerve regeneration.

Keywords:*Treadmill exercise, Wallerian degeneration, Macrophage, Peripheral nerve regeneration.*

1. Introduction

The benefits of physical exercise on neural motor function have been recognized constantly [1].Previous studies report that physical activity promotes adult neurogenesis [2] and neural protection and rescue effect after nerve injury [3]. So, the exercise intervention has been [12]used for the improvement of motor function after nerve injury both in animal models and in clinical therapy [4]. In our previous study, we detected that the treadmill exercise induce the functional recovery after peripheral nerve injury by increased neurotrophic factors [5].If the peripheral nerve injured, nerve fibers distal to the lesion degenerate by the process

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known as Wallerian degeneration, and the neurons reinitiate axonal growth with its attendant metabolic changes. Macrophages are recruited to the injury sites, and along with Schwann cells, contribute to debris clearance. While the distal stump undergoes degeneration, the proximal stump begins regeneration [6]. Wallerian degeneration leads to the removal of axonal and myelin-derived debris, and prepares the environment for regenerating axons grow [7]. A similar process occurs in unmyelinated nerve fibers [8]. As reported previously, wallerian degeneration is crucial for better neuronal regeneration. Recent study report that different monocyte subsets can differentiate into functionally distinct macrophage subsets can differentiate into functionally distinct macrophage subpopulations in vivo [9]. Although further study is needed to clarify about this, there is much evidence of definite macrophage subpopulations is detected in different circumstances [10], or in response to different stimuli [11].It is now known that macrophages can play different roles in inflammation through polarization; M1 macrophages mainly serve “pro-inflammatory” functions of clearing debris and cell, whereas M2 macrophages promote “recovery” and facilitate regeneration of tissues. The macrophage polarization in various inflammatory condition have been a focus of intense research, and the crucial roles of M1/M2 polarization in central nervous system inflammation have been now well-studied. At least in the spinal cord injury, physical exercise appears to facilitate neuroregeneration by favoring M2 polarization in the early phase of injury. However, the roles of macrophage polarization in peripheral nerve injury and exercise have not been studied to date.Activation of the ligand-activated nuclear transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ) exerts beneficial effects through regulating the expression of genes relevant to inflammation, and to the monocyte/macrophages [12]. The pharmacological activation of PPAR-γ using high-affinity thiazolidinedione ligands can enhance IL-4-mediated priming of primary monocytes for differentiation towards an M2 macrophage phenotype in vitro (as measured by phenotypic marker studies involving the M2 marker the Mannose Receptor (MR) [13]. Recent study reports that low-intensity exercise can significantly increase PPAR-γ gene expression and activity in human leukocytes, cells that include circulating mono- cytes. Also, the scavenger receptor CD36, the liver X receptor a (LXR a), and two important LXR a target genes ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1) are upregulated by exercise [14]. So, we hypothesized that low intensity exercise will effect to M2 macrophage by PPAR-γ gene expression and down streams.In the present studies, we tried to find the proper start time point of treadmill exercise after the peripheral nerve injury. So we investigated the macrophages that have pivotal roles in the nerve lesion. M1 type of macrophages doing role for cleaning up damaged nerve debris and M2 type of macrophages have potentials for enhance the regeneration of nerve. So the type of macrophages could be crucial when the Wallerian degeneration processed. To detect the answer of our hypothesis, we compare the experimental results with early started exercise group and later started exercise group and sedentary no exercise group. Our data support the notion that later started treadmill exercise may provide the benefits to the nerve regeneration and functional recovery after PNI.

2. Material and methods

2.1. Animal

Experiments were conducted using male wild type mice on a C57Bl/6J background. C57Bl/6J wild type mice were purchased from Jackson Laboratories. The animals were 4-6

weeks old, weights between 20~30gram, was used for this study. Animals were randomized grouping as Control group (Con), No Exercise group (No Ex), Early started Exercise group(3d Ex) and Later started Exercise group(14th Ex). Each group was consisted of 10 animals.

2.2. Median nerve injury and postoperative care

All surgeries done under deep anesthesia with isoflurane and also aseptic conditions. Briefly, anesthetized animals median and ulnar nerve was exposed and cut with sharp scissors. The cut median nerve was then repaired with common suture method. But the cut ulnar nerve was tied with sterile silk to prevent the regeneration and the skin stapled with surgical clamps.

2.3. Low intensity aerobic treadmill running program

After the operation, all mice had three day rest. Thus, the treadmill exercise started from three days after the operation. Exercise program consisted of 10 min of continuous running at a 10 m/min speed with 5 min warm up(6 m/min) and 5 min cool down(6 m/min) with no incline, applied five days per week for three weeks as described previously, with minor modifications. Mice subjected to median nerve transection were able to run at 10 m/min for 10 minutes continuously beginning three days after surgery, despite the loss of grip strength power.

2.4. Electromyography (Motor nerve conduction velocity)

Electrophysiologic studies were performed on the median nerve using techniques standard for our laboratory. After the animals were anesthetized with isoflurane(flow rate 2 L/min), the compound muscle action potential (cMAP) was recorded from the ventrum of the fore arm. Briefly, motor nerve conduction velocity was determined using a noninvasive procedure in the median nerve posterior conducting protocol in a temperature controlled environment. The right median nerve was stimulated first at the pectoralis minor proximal area and then at the deltoid tuberosity. Stimulation consisted of single 0.2-ms supramaximal (8 V) pulses through a bipolar electrode (Disposable Subdermal Needle Electrode; CareFusion, Middleton, WI). The motor nerve conduction velocity were measured in meters per second.

3. Results

3.1. Later started exercise induced the cMAP and distal latency recovery in the regenerating nerve

Before we sacrificed the animals, we had done EMG test with all groups. cMAP(compound muscle action potential) and time delay is electro-biological marker of nerve regeneration. In pre OP nerves, all of groups have no difference. But, after the experiment, we could see the differences between the exercised group and sedentary no exercised group. Both of EMG items, cMAP and time delay, are increased in exercised group than sedentary no exercised group. Also we can see the difference between the early started group and later started group. Later started exercise group shown better results of regeneration than early started exercise group. With these results we can conclude that later started treadmill exercise enhance the nerve regeneration.

3.2. Later started exercise induces the morphologic recovery in the nerve regeneration

We analyzed electron micrograph(EM) pictures after took pictures using Zeiss Axiophot microscope(Hitech Instruments Inc, MD, US.) and openlabimprovision 5.5.2 software(Image Processing & Vision Company Limited. US.). We detected that more axon numbers in exercised group than no exercised group. They show statistical significance between the control group and exercised group and no exercised group and exercised group either. With this result we can conclude that the treadmill exercise effect to axon regeneration. We also detected that better G-ratio are shown in exercised group than no exercised group. They show statistical significance between the control group and exercised group and no exercised group and exercised group either. With this result we can presume that the treadmill exercise effect to axon/fiber ratio. So, our results support the previous study that exercise enhances the axon regeneration following peripheral nerve injury.

3.3. Later started treadmill exercise increase M2 type macrophages in injured nerve region

We checkedM1 type macrophage's bio-markers(iNOS, IFN-r, TNF-a), M2 type macrophage's bio-markers(Arg1, IL=10, Trem2) and PPAR-γ's mRNA expression level using Real Time PCR analysing methods. We detected that increased M2 type macrophage's bio-markers(Arg1, IL=10, Trem2) and PPAR-γ expression level in exercised group compare with no exercised group. Also we had detected higher expression level in later started treadmill exercise group than early started exercise group. Previous study report that the expression of PPAR-γ induces M2 type macrophages in human atherosclerotic lesions[15].With this reference we can presume that increased PPAR-γ by treadmill exercise in injured nerve induces M2 type macrophages in later started treadmill exercise group and M2 type macrophages effect to nerve regeneration. In short, with this result we can presume that exercise increase M2 type macrophages and effect to regeneration by PPAR-γ signal in later started treadmill exercise group.

4. Conclusion

It has been a hot issue that the proper starting time point of therapeutic exercise in the rehabilitation exercise field. But, we still have no biological or experimental evidences about that. Early period of nerve regeneration, Wallerian degeneration and exercise effect on regenerating peripheral nerve models is not studied enough yet. So we investigated the influence of in early started(3rd day) treadmill exercise and later started(14th day) treadmill exercise on macrophages that are doing the role cleaning damaged nerve debris and also have potentials for betterWallerian degeneration. To investigate the recruited macrophage, we did RT-PCR and detected the expression level of M1 type macrophage's bio-markers(iNOS, IFN-r, TNF-a), M2 type macrophage's bio-markers(Arg1, IL=10, Trem2). Also, we did Electromyograph(EMG) test to check the regeneration degree of nerve. Our result shows the better morphometrics (axon number and g-ratio) and Electrophysiological(cMAP and distal latency) recovery in later starting(14th day) exercised group than early starting(3rd day) exercised group. With our results, we have concluded that later started(14th day) treadmill exercise induced M2 type macrophages during the Wallerian degeneration after peripheral nerve injury. So it has more beneficial effect than early started(3rd day) exercise by induce the M2 type macrophages. In the present study, we define that later started(14th day) therapeutic exercise interventions have better beneficial effect to peripheral nerve

regeneration. But we want to say that overloaded over exercise can harmful to any time point of nerve regeneration.

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