Effects of Insulin-Like Growth Factor 1 on Muscle Atrophy and Motor Function in Rats with Brain Ischemia

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Abstract

Although insulin-like growth factor 1 (IGF 1) has been used in immobilized muscles to prevent muscle atrophy, its effects on muscle atrophy after brain ischemia are not known. This study aimed to determine the effects of IGF 1 on preventing muscle atrophy in rats with brain ischemia. Middle cerebral artery occlusion (MCAO) was used to induce the brain ischemia. In the first part of the study, rats were assigned to sham control, ischemic control, and ischemia with different dosages of IGF 1 injection groups to determine the optimal dosage of IGF 1 on preventing muscle atrophy after brain ischemia. In the second part of the study, rats were assigned to sham control, ischemic control, ischemia with IGF 1, or with IGF 1 receptor inhibitor (AG1024) injection groups to determine the specificity of IGF 1 on preventing muscle atrophy after brain ischemia. IGF 1 or AG1024 was injected locally to calf muscles and anterior tibialis (TA) starting from one day after brain ischemia and injections were carried out every other day for 4 times. Muscle weight and myosin heavy chain (MHC) expression in both red (red gastrocnemius and soleus) and white (white gastrocnemius and TA) muscles were significantly decreased after brain ischemia. With at least moderate-dosage (200 ng/100 µl PBS) IGF 1 injection, the muscle weight and MHC protein could be restored in both red and white muscles resulting in better motor performance. However, the high-dose injection of IGF 1 (400 ng/100 µl PBS) did not result in further effects. IGF 1 increased the expression of p-Akt, but such effects were prevented by AG1024 resulting in muscle atrophy and poor motor function. In conclusion, peripheral application of IGF 1 not only prevented muscle atrophy but also enhanced motor function in rats with brain ischemia. The IGF 1-induced PI3K/Akt pathways are important for preventing muscle atrophy induced by brain ischemia.

Key Words: insulin-like growth factor 1, muscle atrophy, motor function, brain ischemia

Introduction

Muscle atrophy is found in spinal cord-isolated and hind-limb suspended animals (12, 31). In human studies, prolonged bed-rest also causes the decrease in muscle protein synthesis (10). Disuse of muscle or decrease of stimulation from motor neurons may both contribute to muscle atrophy (3). After brain ischemia, dysfunction of upper motor neuron and decrease in muscle activation also cause muscle atrophy. There are about 60% of survivals from cerebral vascular disease with muscle atrophy or weakness on the affected limbs (15). Moreover, in patients with severe cerebral vascular disease, muscle atrophy has been
noticed even in the early recovery stage (13). Muscle atrophy after cerebral vascular disease may lead to further long-term disabilities such as walking dysfunction, falls and even bone fracture (23).

Resisted exercise training is one intervention to restore muscle mass and to prevent muscle atrophy. The contractile force, protein synthesis rate and the somatosensory function of the elderly have all been shown to improve after three months of progressive resistance exercise training (2, 30). However, in patients with severe cerebral vascular disease, resisted exercise may not be feasible, especially in the early stage. Therefore, it is important to develop other possible measures of intervention to prevent muscle atrophy at early recovery stages after cerebral vascular disease.

Animal studies have shown that insulin-like growth factor 1 (IGF 1) has effects on preventing muscle atrophy, and even restoring muscle mass in atrophic muscle (8, 24, 31). The administration of IGF 1 was found to decrease the muscle protein degradation rate and enhance the muscle protein synthesis to maintain contractile protein properties (31). In muscle injury animal model, IGF 1 improved the recovery of muscle (25). In normal animals, the intervention of IGF 1 resulted in not only muscle hypertrophy but also improvement in motor functions (16). These studies suggested that IGF 1 has effects on protecting skeletal muscles from peripheral-induced muscle atrophy, and that IGF 1 also participates in neuromuscular control demonstrated in normal animals. However, effects of IGF 1 on muscle atrophy after brain ischemia remains to be investigated. The present study aimed to investigate possible protective effects of IGF 1 on muscle atrophy and motor function after brain ischemia.

Materials and Methods

**Experiment I**

To determine the optimal dosage of IGF 1 in preventing muscle atrophy after brain ischemia, adult Sprague-Dawley rats (8 weeks of age, body weight 300-350 g) were used. All experiments were performed in accordance with the guidelines of Laboratory Animal Care (NIH publication), and all experimental procedures were approved by the Institutional Animal Care and Use Committee of National Yang-Ming University, Taipei, Taiwan (IACUC-941147). The rats were housed in a temperature-controlled room (22 ± 1°C) with daily artificial illumination for 12 h (07:00 AM-07:00 PM). All animals were given food and water *ad libitum*. Rats were randomly assigned to one of the five groups (n = 8 for each group): SC group: sham control; MI group: middle cerebral artery occlusion (MCAO) control; MI-low group: MCAO with low-dosage (100 ng/100 µl PBS) IGF 1 injection; MI-mod group: MCAO with moderate-dosage (200 ng/100 µl PBS) IGF 1 injection; and MI-high group: MCAO with high-dosage (400 ng/100 µl PBS) IGF 1 injection. Rats in the SC and MC groups received 100 µl PBS injection as injection control at comparable time as the MI groups.

**Brain Ischemia**

Brain ischemia was induced by MCAO procedures. Brain ischemia was conducted under chloral hydrate anesthesia (with single 0.5 g/kg i.p. bolus in 1 ml of saline provided anesthesia lasting at least 2 h). Briefly, a 2-cm burr hole was drilled at the junction of the zygomatic arch and the squamous bone, followed by a 2-cm vertical skin incision midway between the right eye and ear, and split the temporalis muscle. The right middle cerebral artery (MCA) trunk was ligated immediately above the rhinal fissure with a 10-0 suture. Complete interruption of blood flow was confirmed under an operating microscope. Both the common carotid arteries (CCAs) were then occluded using nontraumatic aneurysm clips. After the predetermined duration of ischemia (60 min), the aneurysm clips and the suture were removed from both the CCAs and the MCA. Restoration of blood flow in all 3 arteries was observed directly under the microscope. During the period of surgery, the rectal temperature was monitored and maintained at 37.0 ± 0.5°C by using a heating blanket with an electronic temperature controller (WATLOW 050100C1, Bowdoinham, USA). After the incision was sutured and recovered from anesthesia, the rats were returned to their cages and allowed free access to food and water (26, 28, 29). Neurological examination was performed at 24 h after MCAO by using a neurological grading system with a 5-point scale (0-4); the grades were as follows: 0 = no apparent deficit; 1 = left forelimb flexion; 2 = decreased grip of the left forelimb when the tail is
pulled; 3 = spontaneous movement in all directions, but left circling only if pulled by tail; and 4 = spontaneous left circling (18). Rats that did not show any neurological signs (score 0) after MCAO were excluded from the study.

**Intramuscular Injection of IGF 1 and AG1024**

Rats in MI-low, MI-mod, and MI-high groups received intramuscular injection of different dosages of IGF 1. The low-, moderate- and high-dosage IGF 1 received intramuscular injection of IGF 1 receptor inhibitor (3-Bromo-5-t-butyl-4-hydroxy-benzylidenemalonitrile, AG1024, Invitrogen, catalog number I8779) was dissolved in 0.1 M PBS buffer containing 0.2% bovine serum albumin (BSA) at pH 7.4~7.6. The AG1024 powder (Invitrogen, catalog number PHZ1173) was freely dissolved in the DMSO solution and then diluted with 0.1 M PBS buffer to adjust the concentration to 30 µg per 100 µl. The AG1024 solution was stored at -20°C and avoided light. IGF-1 or AG1024 were injected locally in left calf muscles and anterior tibialis muscle beginning at the second day post MCAO and then injected every other day for a total of 4 times. Rats in the SC and MC groups received intramuscular injection of 100 µl 0.1 M PBS buffer to the left calf muscles and anterior tibialis muscle at the comparable time of MI groups.

**Muscle Weight Measurements**

Seven days after MCAO, rat was anaesthetized by chloral hydrate anesthesia (with single 0.5 g/kg i.p. bolus in 1 ml of saline provided anesthesia lasting at least 2 h) at 1 h after the final injection. The gastrocnemius, soleus and anterior tibialis muscles in the affected (left) side were carefully removed. After cleaning the connective tissues, the muscle samples were weighted. The red- and white-gastrocnemius portions were separated because these two portions consist of different types of muscle fiber, slow- and fast-type, respectively. The predominant slow-type fibers of gastrocnemius muscle are located near the tibial bone part (medial portion) and the predominant fast-type fibers of gastrocnemius muscle are located near the subcutaneous part (lateral portion) (17, 19). In each muscle, a 0.5-cm thick muscle belly was cut from the midline and used as the muscle sample. The muscle samples were then quickly frozen by liquid nitrogen and stored in -80°C. The muscle weight was normalized by body weight.

**Muscle Protein Measurements**

Muscle samples were lysed in lysis buffer containing 0.02 M HEPES, 0.25 M sucrose, 0.2% sodium azide, and 0.2 mM phenylmethysulfonyl fluoride (PMSF). The lysis buffer was added cocktail inhibitor in a dilution of 1:100 in addition. The lysates were centrifuged at 12,500 × g for 30 min at 4°C. The concentration of protein in the supernatant was determined using the Bradford-red protein assay. After adding the sample buffer containing 0.1 M Tris-Cl (pH 6.8), 25% glycerol, 2% SDS, 0.02% bromophenol blue and 5% β-mercaptoethanol, the sample was boiled for 10 min. A total of 50 µg of protein in each sample was resolved in 8% SDS-polyacrylamide gel and then transferred onto polyvinylidene fluoride membrane (Millipore Corp., Billerica, MA, USA). The membrane was blocked with 0.1% Tween 20/Tris buffered saline (TBST) containing 5% nonfat milk at room temperature for 60 min, and then incubated with primary antibodies, including anti-myosin heavy chain-slow type or fast type (Sigma, USA), anti-phosphorylated Aktser473 (p-Aktser473) (Millipore Corp., Billerica, MA, USA) and anti-total Akt (t-Akt) antibodies (Millipore Corp., Billerica, MA, USA), and the internal control anti-beta actin antibody (Sigma, USA) at a dilution of 1:2000 (MHC), 1:500 (p-Aktser473 and t-Akt) and 1:4000 (beta-actin) with TBST at 4°C overnight. After washing three times, the membrane was incubated for 1 h with horseradish peroxidase-conjugated goat-anti-mouse or anti-rabbit IgG secondary antibodies (Millipore) diluted 1:6000 with TBST at room temperature. The signals were visualized using a western blot chemiluminescence reagent plus (ECL plus; Amersham Biosciences Co. Ltd., Kowloon, HK). To quantify the amount of proteins after blotting, the band density of immunoblots was measured using an image analysis software (ImageQuant; Amersham Biosciences Co. Ltd., Kowloon, HK). The ratios of densitometry data to that of t-Akt of each muscle were calculated and were expressed as percentages to the SC group.

**Motor Function Measurement**

The parallel-bar-crossing test was performed to test hind-limb coordination (9). The parallel bars were 2 parallel acrylic rods (diameter, 1.0 cm; length, 110 cm) with an inter-rod distance of 2.5 cm that were connected to platforms at each end (15 × 50 cm²). In each trial, rats were placed on a platform and encouraged to walk on the parallel bars for 1 min. The instances when both hind paws were placed on 1 row, when a hind paw slipped over the rod, and when the rats fell or swung from the rods were recoded as
errors. The errors made per meter in 1 min (errors per meter) were used for analysis. Fewer errors made in this motor function test indicated better hind-limb function. This motor function was tested at 24 h after MCAO and 1 h after the final injection.

**Statistic Analysis**

The data of muscle weight to body weight ratio, expression of muscle proteins and motor performance were presented as means ± standard error of means. Differences in muscle weight to body weight ratio and muscle proteins among groups were examined by using one way analysis of variances (one way ANOVA) with post hoc turkey test. The significant level was set at $P < 0.05$. The differences in motor function were first analyzed by the two way ANOVA analysis (groups × times). Turkey test was employed for post-ANOVA analysis between groups. Paired Student’s $t$ test was employed to test the time effect within each group.

**Results**

In this study, the median of the neurological score measured at 24 h after MCAO were 3 (range: 1-3), 2 (range: 2-3), 3 (range: 2-3), 3 (range: 1-3) and 2 (range: 1-3) in the MC, MI-low, MI-mod, MI-high and MAgI groups, respectively. There was no statistical difference among groups and no rat died during the experimental period.

*Muscle Weight to Body Weight Ratio*

The results of muscle weight to body weight ratio in Experimental I are shown in Fig. 1. Seven days after MCAO, the MC group showed a significant decrease in muscle mass in affected gastrocnemius ($P < 0.01$), anterior tibialis ($P < 0.01$) and soleus ($P < 0.01$) muscles when compared with the SC group. These data indicate that after brain ischemia, the muscle mass decreased in the affected hind-limb even at early stage.

In the MI-low group, the muscle weight to body weight ratios of gastrocnemius and anterior tibialis muscles were still significantly less than those of the SC group ($P < 0.01$ in gastrocnemius and anterior tibialis muscles). But, low-dosage IGF 1 (100 ng/100 µl PBS) seemed adequate to prevent soleus muscle atrophy.

In the MI-mod and MI-high groups, the muscle weight to body weight ratios of gastrocnemius, anterior tibialis and soleus muscles were still significantly higher than those of the MC group ($P < 0.01$ in gastrocnemius and anterior tibialis muscles) but there was no significant difference from those of the SC group. There was no significant difference between the MI-mod and MI-high groups in muscle weight to
body weight ratio of gastrocnemius, anterior tibialis or soleus muscles. Our data indicate that at least moderate-dosage IGF 1 injection for 4 times can effectively prevent muscle atrophy induced by brain ischemia, and higher dosages did not exert further effects.

The results of muscle weight to body weight ratio in Experiment II are shown in Fig. 2. Similar to the results in Experiment I, with IGF 1 injection, the muscle weight to body weight ratios of gastrocnemius, anterior tibialis and soleus muscles were significant higher than those of the MC group ($P < 0.01$ in gastrocnemius, anterior tibialis and soleus) but there were no significant differences from those of the SC group. However, the muscle weight to body weight ratios of gastrocnemius ($P < 0.01$), anterior tibialis ($P < 0.01$) and soleus ($P < 0.01$) muscles in the MAgI group were significantly decreased when compared with the SC and MI groups but not significant from those of the MC group. These results indicate that IGF 1 signaling is essential to prevent muscle atrophy after brain ischemia.

**Muscle Protein Expressions**

The expression of the muscle contractile protein, myosin heavy chain (MHC), in Experiment I is shown in Figs. 3 and 4. The expression of MHC-slow type in red-gastrocnemius muscle ($P < 0.01$) and MHC-fast type in white-gastrocnemius ($P < 0.01$) and anterior tibialis muscles ($P < 0.01$) in the MC group were significantly decreased when compared with the SC group.

The expression of MHC-slow type in red-gastrocnemius muscle ($P < 0.01$) and MHC-fast type in white-gastrocnemius ($P < 0.01$) and anterior tibialis muscles ($P < 0.01$) in the MI-low group were still significantly lower than in the SC group (Figs. 3 and 4). However, the expression of MHC-slow type in soleus muscle in the MI-low group showed no difference from the SC group (Fig. 3).

The expression of MHC-slow type in red-gastrocnemius and soleus muscles and MHC-fast type in white-gastrocnemius and anterior tibialis muscles in the MI-mod and MI-high groups were significant higher from those of the MC group ($P < 0.01$ in red gastrocnemius, white gastrocnemius, anterior tibialis, and soleus) but not significantly different from those of the SC group (Figs. 3 and 4). There was no significant difference between the MI-mod and the MI-high groups in MHC-slow and MHC-fast types. These results indicate that intramuscular local injection with at least moderate-dosage of IGF 1 to affected hind-limb can prevent contractile protein degradation after brain ischemia.

The expression of MHC in Experiment II is shown in Figs. 5 and 6. Similar to the results in Experiment I, with IGF 1 injection, the expression of MHC-slow type in red-gastrocnemius and soleus muscles and MHC-fast type in white-gastrocnemius and anterior tibialis muscles were significant higher
than those of the MC group ($P < 0.01$ in red gastrocnemius, white gastrocnemius, anterior tibialis, and soleus) but no significant difference to those of the SC group (Figs. 5 and 6). However, the expression of MHC-slow type in red-gastrocnemius ($P < 0.01$) and soleus muscles ($P < 0.01$) and MHC-fast type in white-gastrocnemius ($P < 0.01$) and anterior tibialis muscles ($P < 0.01$) in the MAgI group were significantly decreased when compared with the SC and MI groups but no significant from those of the MC group (Figs. 5 and 6). These data show that IGF 1 signaling is essential to prevent muscle contractile protein breakdown after brain ischemia.

Furthermore, the expression of p-Akt$^{ser473}$ in the red-gastrocnemius, white-gastrocnemius and anterior tibialis muscles were all significantly decreased in the MC group when compared with the SC group (Fig. 7). However, the expression of p-Akt$^{ser473}$ in the soleus muscle did not decrease significantly in the MC and MAgI groups. With IGF 1 injection (the MI
The expression of p-Akt ser473 in the red-gastrocnemius, white-gastrocnemius, anterior tibialis, and soleus muscles were significant increased when compared with the MC group (P < 0.01) but not significantly different from the SC group. Only the expression of p-Akt ser473 in the soleus muscle showed significant increase in the MI group when compared with the SC group (P < 0.05). The expression of p-Akt ser473 in the red-gastrocnemius (P < 0.01), white-gastrocnemius (P < 0.01), anterior tibialis muscles (P < 0.01) and soleus muscles (P < 0.01) in the MAgI group was significantly decreased when compared with the MI group but no significant from those of the MC group (Fig. 7). These results indicate that PI3K-Akt signaling induced by exogenous IGF 1 maybe involved in the prevention of muscle atrophy after brain ischemia in the early stage. Take together, the application of IGF
1 after brain ischemia is effective in preventing muscle atrophy, and the underlying mechanisms may, at least in part, act through the PI3K-Akt signaling.

**Motor Function**

The result of motor performance in the parallel-bar-crossing test is shown in Fig. 8. According to two way ANOVA, a significant time effect was noted \([F = 18.5, P < 0.01]\), and an interaction between group and time \([F= 3.1, P < 0.05]\) was also noted. The results of post hoc analysis indicated significant motor impairment in rats after brain ischemia \((P < 0.01)\). With moderate- or high-dosage IGF 1 injection, the motor function of brain ischemic rats improved significantly, although such improvement could not reach the level as demonstrated in the SC group \((P < 0.01)\). No significant differences in the motor function between the MI-mod and the MI-high groups were noted. However, the result in motor performance in the MAgl group showed significant difference from that in the MI group \((P < 0.01)\), but not significantly different from the MC group. Our results indicate that application of IGF 1 not only prevents muscle atrophy after brain ischemia but also enhances the recovery of motor function after brain ischemia.

**Discussion**

In the present study, our data demonstrated that brain ischemia could cause muscle protein degradation in affected hind-limb within one week. Our results further indicated that intramuscular IGF 1 injection at least 200 ng/100 µl PBS for 4 times within the first week after brain ischemia could prevent loss of muscle mass and degradation of muscle contractile protein. In addition, the motor function was significantly improved. Furthermore, the p-Akt signaling in affected muscles were restored after IGF 1 injection. Thus, our results indicated that IGF 1 application in the acute stage after brain ischemia could not only prevent muscle atrophy but also enhance motor
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function recovery, and the re-activation of PI3K/Akt signaling pathway may participate in these protecting effects of IGF 1.

Choe et al. showed that 7 days after brain ischemia, the weight of affected gastrocnemius muscle was significantly less than that of the control group, but distribution of the slow and fast muscle fiber did not differ significantly from that in the control group (3). In the present study, we separated the red and white fiber portions of the gastrocnemius muscle to examine the expression of slow- and fast-type muscle fibers, and our results showed that the expression of slow and fast muscle fibers in affected gastrocnemius muscle measured at 7 days after brain ischemia were both significantly decreased. Separation of different portions of gastrocnemius muscle in our study allowed determination of changes in the different muscle fiber types of the gastrocnemius muscle. In addition, our data demonstrated that muscle weight to body weight ratios in the affected soleus and anterior tibialis muscles measured at 7 days after brain ischemia were significantly less than that in the SC group, and the expression of slow and fast muscle fibers was also significantly decreased as the result of brain ischemia. Although the MHC slow-type in red muscles (red gastrocnemius and soleus) and MHC fast-type in white muscles (white gastrocnemius and anterior tibialis) were both significantly decreased in the affected hindlimb after 7 days post-brain ischemia, it seemed that muscles containing fast-twitch fibers were more easily atrophied. Our data showed that MHC fast-type in the anterior tibialis muscle was more observably decreased than MHC slow-type in the soleus muscle 7 days after brain ischemia. It has been shown in normal rats that about 90% of muscle fiber distribution in soleus muscle was slow-type and about 80% in anterior tibialis muscle was fast-type (3). However, whether the difference in muscle type distribution influences the rate of brain ischemia-induced muscle atrophy and the underlining mechanisms remain to be in-

![Fig. 6. Expression of myosin heavy chain, fast type (MHC-fast type) in the white gastrocnemius and anterior tibialis muscle in experiment II. IGF 1 and AG1024 were injected locally in left calf muscles and anterior tibialis muscle beginning on the second post MCAO and then injected every other day for total 4 times. AG1024 was injected 30min before IGF 1 injection in the MAgI group. A: Expression of MHC-fast type among groups (n = 8 for each group). All data are based on the ratio of MHC-slow type densitometry data to that of β-actin. The expression of MHC-fast type in MC, MI, and MAgI groups was presented as percentage to that in the SC group. B: Western blot in white-gastrocnemius muscle among groups (n = 8 for each group). C: Western blot in anterior tibialis muscle among groups (n = 8 for each group). **, P < 0.01 vs. SC group; ##, P < 0.01 vs. MC group; ++, P < 0.01 vs. MI group. SC: sham control; MC: MCAO control; MI: MCAO with IGF 1 injection (200 ng per injection); MAgI: MCAO with AG1024 injection (30 µg per injection) 30 min before IGF 1 application.](image-url)
In our study, low-dosage (100 ng/100 µl PBS) IGF 1 injection did not have effects on preventing muscle atrophy in gastrocnemius, anterior tibialis muscles as indicated by muscle weight and the MHC protein expressions. However, we have noted that the soleus muscle seemed to respond to this low-dosage of IGF 1. It has been shown in normal rats that about 90% of muscle fiber distribution in soleus muscle was slow-type (3). The difference in fiber type distribution in different muscles may contribute to the effect of IGF 1 in reducing muscle atrophy. However, the underlying mechanisms still need further study.

Our results showed that the both moderate- and high-dosage of IGF 1 injection for 4 times could prevent muscle atrophy in gastrocnemius, anterior tibialis and soleus muscles after brain ischemia. However, high-dosage IGF 1 injection did not result in further protective effects on muscle atrophy when compared with the moderate-dosage. This result may indicate that there is a ceiling effect for muscle response to IGF 1.

It has been known that when IGF 1 binds to its receptors on the cell membrane, the downstream insulin receptor substrates 1 and 2 (IRS 1 and IRS2) are activated. The activated IRS 1 then activates the phosphatidyl-inositol-3 kinase (PI3K) and mitogen-activated protein kinase (MAPK). Subsequently, PI3K

Fig. 7. Expression of p-AktSer473 in muscles. IGF 1 and AG1024 were injected locally in left calf muscles and anterior tibialis muscle beginning on the second post MCAO and then injected every other day for 4 times. AG1024 was injected 30 min before IGF 1 injection in the MAgI group. A: Expression of p-AktSer473 among groups (n = 8 for each group). All data are based on the ratio of p-AktSer473 densitometry data to that of total Akt (t-Akt). The expression of p-AktSer473 in MC, MI and MAgI groups was presented as percentage to that in the SC group. B: Western blot in muscles among groups (n = 8 for each group). *, P < 0.05, **, P < 0.01 vs. SC group; ##, P < 0.01 vs. MC group; ++, P < 0.01 vs. MI group. SC: sham control; MC: MCAO control; MI: MCAO with IGF 1 injection (200 ng per injection); MAgI: MCAO with AG1024 injection (30 µg per injection) 30 min before IGF 1 application.
activates the protein kinase B (Akt) which is known as a regulator for cell survival (1, 6, 27). The PI3K/Akt pathway then regulates the expression of glycogen synthase kinase 3β (GSK 3β), mammalian target of rapamycin (mTOR), P70⁶⁶k and the 4EBP-1 signaling pathway which are all suggested to be key factors to promote tRNA functions, to regulate protein synthesis and to inhibit cell apoptosis (5, 20). A previous study indicated that IGF 1 induced skeletal myotube hypertrophy by the PI3K/Akt/mTOR and PI3K/Akt/GSK3 pathways (21). Another study showed that conditional activation of Akt in adult skeletal muscles induced rapid hypertrophy (14). Therefore, IGF 1 can enhance the activation of PI3K/Akt pathway to promote muscle growth. In addition, some studies have reported that IGF 1-mediated activation of PI3K/Akt signaling can prevent the activation of muscle atrophy genes, such as the MAFbx and MuRF 1 genes, that could increase rates of protein breakdown (4, 7, 11, 22, 24). According to the results from these studies, IGF 1 not only promoted muscle protein synthesis but also inhibited the degradation of muscle proteins, thus, protecting muscles from atrophy after brain ischemia. In our study, we noted that MHC and p-Akt level were significantly decreased after brain ischemia, but expressions of the MHC and p-Akt level were restored in rats that received IGF 1 injection. These results indicated that the balance between protein synthesis and protein degradation in these muscles was disturbed after brain ischemia and could be partially re-balanced by IGF 1 injection. Furthermore, we used the IGF 1 receptor inhibitor to determine the specificity of IGF 1 and its downstream PI3K/Akt signaling pathway in preventing muscle atrophy after brain ischemia. Our results showed that both MHC and p-Akt level were significantly decreased in the Magl group. These results confirm that IGF1/PI3K/Akt signaling is essential to prevent muscle atrophy. Although we did not examine the protein synthesis or degradation rates of the affected muscles in the present study, the results in p-Akt level protein expression suggest that the protective effects of IGF 1 on muscle atrophy after brain ischemia were at least partly due to the activation of IGF 1 signaling in these muscles.

The parallel bar crossing test was used to examine the hind-limb function in the present study. Our results showed that motor performance was significantly impaired in rats after brain ischemia. The impairment in motor function after brain ischemia was usually due to damages in the motor control areas including the pre-motor, primary motor and somatosensory cortices, which were all involved in the territory of middle cerebral artery. The muscle contractile ability is also involved in motor functions. Our results showed that the MHC proteins in muscles were significantly decreased in brain ischemic rats, therefore, the damage in the central nerve system and the decrease in the expression of the muscle contractile protein may both contribute to the impairment of motor function. After receiving IGF 1 injection, rats improved significantly in their motor performance. The improvement of motor function was also associated with the restoration of muscle MHC proteins. Our results also showed that IGF 1 increased the expression of p-Akt level in all affected muscles. These results may indicate that IGF 1 promotes muscle protein synthesis to restore the MHC levels, and to enhance the recovery of the motor function. However, the motor function in rats that received IGF 1 injection was not as good as that in the SC group indicating that the motor function was also controlled by the central nervous system. Thus, the improvement in motor function as shown in the present study may not only due to restoration of muscle contractile proteins.

Our results indicated that muscle mass and proteins in the affected hind-limb were significantly decreased with impaired motor function within one week after brain ischemia. With at least certain dosage of IGF 1 injection (as 200 ng/100 µl PBS for 4 injections in the present study), the muscle proteins...
could be restored and resulted in better motor performance. However, there seems to be a ceiling effect of IGF 1 in preventing muscle atrophy after brain ischemia. Our data also suggest that the PI3K/Akt signaling pathway induced by IGF 1 is essential to prevent muscle atrophy after brain ischemic injury. The improvement in motor function after IGF 1 application may partly be due to the restoration of muscle contractile ability.

Acknowledgments

This study was supported by NSC95-2314-B-010-041-MY3 from the National Science Council of the Republic of China.

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