

Interaction between HSP 70 and iNOS in skeletal muscle injury and repair

Kijeong Kim*

School of Exercise & Sport Science, College of Natural Sciences, University of Ulsan, Ulsan, Korea

Muscle injuries are frequently occurred in various sports. The biological process and mechanism of muscle repair after injury are well known through the many studies. This study aimed at presenting heat shock protein and nitric oxide synthase are to respond to muscle damage and repair. This section discusses the results obtained through many articles. Heat shock proteins (HSPs) are considered to play an essential role in protecting cells from damage, preparing them to survive on new environmental challenges. In addition, exercise-induced changes such as heat shock, oxidative, metabolic, muscular, and cytokine stress seem to be responsible for the HSP response to exercise. Also, inducible ni-

tric oxide synthase (iNOS) generates nitric oxide (NO) for prolonged period and causes pathophysiological effects. Furthermore, iNOS is involved in processes such as cell injury, wound repair, embryogenesis, tissue differentiation, and suppression of tumorigenesis. In conclusion, the inhibition of HSP 70 on caspase-3 and apoptosis is associated with its inhibition on iNOS that leads to less NO production.

Keywords: Muscle injury, Repair, Heat shock protein 70, Inducible nitric oxide

INTRODUCTION

Muscle injuries are one of the most common injuries occurring in sports, with an incidence varying from 10% to 55% of all the sustained injuries (Beiner et al., 2001). Muscle injuries are caused by contusion, strain, or laceration (Garrett, 1990). Muscle lacerations are the most uncommon of the muscle injuries occurring in sports, as more than 90% of all sports-related injuries are either contusions or strains (Järvinen et al., 1993). A muscle contusion occurs when a muscle is subject to a sudden, heavy compressive force, such as a direct blow to the muscle. This kind of muscle trauma typically takes place in contact sports, whereas sprinting and jumping are the most common activities that are associated with muscle strains (Garrett, 1990). In strains, an excessive tensile force subjected onto the muscles leads to the overstraining of the myofibers and consequently to a rupture near the myotendinous junction (MTJ). Muscle strains typically concern the superficial muscles working across 2 joints, such as the rectus femoris, semi-

tendinosus, and gastrocnemius muscles (Kalimo et al., 1997).

BIOLOGICAL PROCESS OF MUSCLE REPAIR

Although the myofibers are generally considered to be irreversibly postmitotic, the marked regenerative capacity of the skeletal muscle is guaranteed by an intrinsic mechanism that restores the injured contractile apparatus. Accordingly, a pool of undifferentiated reserve cells, called the satellite cells, is set aside, underneath the basal lamina of each individual myofiber (Kalimo et al., 1997), during the fetal development. In response to injury, these cells first proliferate, then differentiate into myoblasts, and finally join with each other to form multi-nucleated myotubes (Hurme et al., 1991). The newly formed multi-nucleated myotubes then fuse with the part of the injured myofiber that has survived following the initial trauma (Hurme et al., 1991). Eventually, the regenerating parts of the myofibers acquire their mature form

*Corresponding author: Kijeong Kim
School of Exercise & Sport Science, College of Natural Sciences, University of Ulsan,
93 Daehak-ro, Nam-gu, Ulsan 44610, Korea
Tel: +82-52-259-2381, Fax: +82-52-259-1696, E-mail: kijeongk@ulsan.ac.kr
Received: September 25, 2015 / Accepted: October 11, 2015

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

with normal cross-striations and peripherally located myonuclei (Hurme et al., 1991). Interestingly, in response to very mild injury (a single, eccentric stretch-induced injury), the satellite cells respond immediately by starting to proliferate, but because of the mildness of the injury and rapid, “intrinsic” recovery of the injured myofibers, the satellite cells activation halt before the myoblasts arise.

In mature skeletal muscles, there are (at least) 2 major populations of satellite cells (Zammit et al., 2004). The “classic” satellite cells, those residing beneath the muscle fiber basal lamina, can be divided into committed satellite cells, which are ready to begin differentiation to myoblasts immediately after the muscle injury, and stem satellite cells, which first undergo cell division(s) before differentiation (Zammit et al., 2004). Through this cell division (proliferation), the stem population replenishes the reserve of satellite cells for the possible future demands of regeneration (Rantanen et al., 1995). Among this population of satellite cells, there is a sub-population of cells that are capable of differentiating beyond myogenic lineages not only into different mesenchymal lineages (Shefer et al., 2004) but also into the neural or endothelial ones (Jankowski et al., 2002).

The satellite cells were presumed to be the only source of myonuclei in muscle repair (Chargé and Rudnicki, 2004). However, recent findings have demonstrated the presence of 2 different populations of multipotential stem cells that can contribute to the regeneration of injured skeletal muscle: non-muscle resident stem cells and muscle resident stem cells (Chargé and Rudnicki, 2004).

Progenitor cells isolated from bone marrow (BM), the neuronal compartment, and various mesenchymal tissues can differentiate into a myogenic lineage. The BM-derived cells not only contribute to the regenerating myofibers in injured skeletal muscles but also replenish the satellite cell pool in the injured skeletal muscles (LaBarge and Blau, 2002). However, it is worth noting that the frequency at which these events occur seems to be very low (even in the injury) when compared with the number of regenerating myoblasts derived from the satellite cells (Grounds et al., 2002). Thus, it is debatable whether the stem cells make a significant contribution to the regeneration of the injured skeletal muscle at all (Grounds et al., 2002).

In addition to the classic satellite cells residing underneath the basal lamina, there is another distinct population of muscle stem cells located extralaminally within the connective tissue of the skeletal muscles (Dreyfus et al., 2004). In response to injury to the skeletal muscles, these cells readily give rise to determined myoblasts and differentiate to myotubes (Chargé and Rudnicki, 2004).

After the cylinders of the old basal lamina have been filled with the regenerating myofibers, the myofibers further extend through the opening on the basal lamina toward the connective tissue scar that has formed between the survived stumps of the myofibers (Kalimo et al., 1997). On both sides of the connective tissue scar, the myofibers of the survived muscle stumps form multiple branches while trying to pierce through the scar separating them (Hurme and Kalimo, 1992). However, after managing to extend only for a relatively short distance, the branches begin to adhere to the connective tissue with the tips at their ends, forming mini-MTJs with the scar. With time, the intervening scar progressively diminishes in size, bringing the stumps in closer adherence to each other (Vaittinen et al., 2002), but it is still unknown whether the stumps of the transected myofibers from the opposing sides of the scar will ultimately ever fuse with each other or whether some form of connective tissue septum remains between them (Vaittinen et al., 2002).

HEAT SHOCK PROTEINS

Strenuous exercise creates physiological stress, disturbs cellular homeostasis, and ultimately induces cellular adaptations. Heat shock proteins (HSP) are considered to play an essential role in protecting cells from stress, preparing them to survive on new environmental challenges including an immune response.

Exercise-induced changes such as heat shock, oxidative, metabolic, muscular, and cytokine stress seem to be responsible for the HSP response to exercise. Acute exercise is obviously able to stimulate the expressions of certain HSP in different cells and tissues including muscle, heart, liver, brain, leukocytes, and plasma. The HSP response due to exercise has been documented in animals as well as the human species (Kiang, 2004).

The extent of the HSP response is dependent on type, intensity, and duration of exercise, environmental conditions, and cell type examined. Training status and gender also have an impact on the HSP response. A large individual variability in induction of HSP expression and the particular HSP being examined have to be considered (Kiang, 2004).

Repeated exercise bouts, regular training, and high ambient temperature induce an adaptation of the HSP response in muscles and immune cells. Measurement of HSP content seems to be an indicator of exercise-related stress situations that may possibly allow conclusions concerning adaptation to training or may even represent a marker to warn one of overtraining in the near future (Kiang, 2004).

In conclusion, exercise and training interfere with HSP in a complex way, which includes up-regulation through acute exercise and sophisticated regulation through regular training. The extent to which this related to the beneficial effects of exercise on the immune system or the occurrence of certain kinds of cancer remains open to debate (Kiang, 2004).

NITRIC OXIDE SYNTHASE

Nitric oxide (NO) production is mediated by nitric oxide synthase (NOS). NOS can be divided into two major categories: constitutive form (cNOS) and inducible form (iNOS). cNOS includes nNOS and eNOS and is Ca^{2+} -dependent, whereas iNOS is not. Both cNOS and iNOS contain a reductase domain and an oxidase domain. However, eNOS has myristonylation and palmitonylation sites, whereas nNOS and iNOS do not. The iNOS promoter has a TATA site and differs in that from the cNOS promoters which are TATA-less promoters. The cNOS promoters are GC rich and are regulated primarily by Sp1 and other members of the Sp1-like family. In contrast, nuclear factor (NF)- κ B plays a crucial role in the regulation of iNOS. In the murine iNOS promoter, the downstream NF- κ B binding site (-76 to -85 bp) seems to be the most important one, however, the upstream NF- κ B site (-974 to -960 bp) also seems to have some functionality and cooperativeness with the downstream site. For the human iNOS promoter, the NF- κ B at -5.8 kb is most critical for cytokine-induced promoter activity, whereas the sites at -5.2, -5.5, -6.1, and -8.2 kb have a cooperative effect (Kiang, 2004).

cNOS is thought to generate low levels of NO at sub-micromolar range for short duration and perform many physiological effects. iNOS generates NO for prolonged period and at local concentrations as high as 1-5 μM and causes pathophysiological effects. Since the chemistry and biological outcome depends on the concentration of NO, the proximity of a biological target to the NO source becomes critical. For example, cells or tissues close to macrophages that produce high levels of NO will be subjected to direct and indirect effects due to the primary and the secondary modes of NO actions. In contrast, if they are far from the NO source, they will experience only direct effects as the primary mode of NO action (Kiang, 2004).

Hemorrhage induces sequential increases in the levels of stress-related proteins c-JUN, cNOS, KLF6, iNOS and then HSP 70 and HIF-1 α . Both cNOS gene and iNOS gene get activated by hemorrhage and they are early response genes, though cNOS responds to hemorrhage earlier than iNOS. HSP 70 and HIF-1 are considered

to be the late response genes and their overexpression probably play a role to control the post-hemorrhage tissue damage.

iNOS has previously been shown to be overexpressed after hemorrhagic shock in rodent lung, human liver, and murine tissues. The binding of Kruppel-like factor 6 (KLF6) to the iNOS promoter increased significantly in cultured cells after chemical hypoxia, heat stress, serum starvation, and phorbol 12-myristate 13-acetate and A23187 ionophore stimulation. Furthermore, both the KLF6 promoter and iNOS promoter have AP-1 binding sites (KLF6: -364 to -371; iNOS: -644 to -650 and -1,225 to -1,231) that probably also bind c-JUN and/or c-FOS. In our study, no c-FOS was detected using immunoblotting analysis. Because iNOS promoters include two AP-1 sites and are involved in processes such as cell injury, wound repair, embryogenesis, tissue differentiation, and suppression of tumorigenesis, the observation that c-JUN overexpression occurs earlier than KLF6 and iNOS overexpression suggests two possibilities. 1) Hemorrhage activates c-JUN, which up-regulates KLF6 expression, leading to iNOS expression; or 2) hemorrhage activates c-JUN, which then directly binds to the iNOS promoter to induce iNOS expression (Kiang, 2004).

INTERACTION BETWEEN HSP 70 AND iNOS

Using immunoprecipitation and immunoblotting analysis, it has been found that HSP 70 forms a complex with iNOS and its transcription factor KLF6 after hemorrhage, probably by iNOS and KLF6 binding to the peptide binding domain of HSP-70. No complex formation is detected between HSP-70 and p53 or Bcl-2 proteins, suggesting that HSP 70 specifically couples to iNOS and KLF6 (Kiang, 2004). Treatment with geldanamycin increases HSP 70 expression and decreases the hemorrhage-induced increase in iNOS expression. The complex formation between HSP 70 and iNOS is still observed. It is possible that the complex formed between HSP 70 and iNOS might decrease the enzymatic activity of iNOS and subsequently decrease NO production. As a result of a low level of NO, the morbid sequelae caused by hemorrhagic shock is markedly diminished (Kiang, 2004).

CONCLUSIONS

Our preliminary data show that up-regulation of HSP 70 by HSP 70 gene transfection to human intestinal cells inhibited the hypoxia-induced increase in caspase-3 activity and reduced apoptosis. Likewise, treatment of human intestinal epithelial cells with

iNOS inhibitors decreased caspase-3 activity and apoptosis. Taking these data together, it can be concluded that the inhibition of HSP 70 on caspase-3 and apoptosis is associated with its inhibition on iNOS that leads to less NO production (Kiang, 2004).

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

This work was supported by the 2015 Research Fund of University of Ulsan.

REFERENCES

- Beiner JM, Jokl P. Muscle contusion injuries: current treatment options. *J Am Acad Orthop Surg* 2001;403:S110-119.
- Chargé SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. *Physiol Rev* 2004;84:209-238.
- Dreyfus PA, Chretien F, Chazaud B, Kirova Y, Caramelle P, Garcia L, Butler-Browne G, Gherardi RK. Adult bone marrow-derived stem cells in muscle connective tissue and satellite cell niches. *Am J Pathol* 2004; 164:773-779.
- Garrett WE Jr. Muscle strain injuries: clinical and basic aspects. *Med Sci Sports Exerc* 1990;22:436-443.
- Grounds MD, White JD, Rosenthal N, Bogoyevitch MA. The role of stem cell in skeletal and cardiac muscle repair. *J Histochem Cytochem* 2002;50:589-610.
- Hurme T, Kalimo H. Activation of myogenic precursor cells after muscle injury. *Med Sci Sports Exerc* 1992;24:197-205.
- Hurme T, Kalimo H, Lehto M, Järvinen M. Healing of skeletal muscle injury: an ultrastructural and immunohistochemical study. *Med Sci Sports Exerc* 1991;23:801-810.
- Jankowski RJ, Deasy BM, Cao B, Gates C, Huard J. The role of CD34 expression and cellular fusion in the regeneration capacity of myogenic progenitor cells. *J Cell Sci* 2002;115:4361-4374.
- Järvinen M, Lehto M. The effect of early mobilization and immobilization on the healing process following muscle injuries. *Sports Med* 1993;15: 78-89.
- Kalimo H, Rantanen J, Järvinen M. Muscle injuries in sports. *Baillieres Clin Orthop* 1997;2:1-24.
- Kiang JG. Inducible heat shock protein 70 kD and inducible nitric oxide synthase in hemorrhage/resuscitation-induced injury. *Cell Res* 2004;14:450-459.
- LaBarge MA, Blau HM. Biological progression from adult bone marrow to mononucleate muscle fiber in response to injury. *Cell* 2002;111:589-601.
- Rantanen J, Hurme T, Lukka R, Heino J, Kalimo H. Satellite cell proliferation and expression of myogenin and desmin in regenerating skeletal muscle: evidence for two different populations of satellite cells. *Lab Invest* 1995;72:341-347.
- Shefer G, Wleklinski-Lee M, Yablonka-Reuveni Z. Skeletal muscle satellite cells can spontaneously enter an alternative mesenchymal pathway. *J Cell Sci* 2004;117:5393-5404.
- Vaittinen S, Hurme T, Rantanen J, Kalimo H. Transected myofibers may remain permanently divided in two parts. *Neuromuscul Disord* 2002; 12:584-587.
- Zammit PS, Golding JP, Nagata Y, Hudon V, Partridge TA, Beauchamp JR. Muscle satellite cells adopt divergent fates: a mechanism for self-renewal? *J Cell Biol* 2004;166:347-357.