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Original Article

Phosphorylation of Heat Shock Protein 27 is Increased by Cast Immobilization and by Serum-free Starvation in Skeletal Muscles

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Abstract. [Purpose] Cast immobilization- and cell starvation-induced loss of muscle mass are closely associated with a dramatic reduction in the structural muscle proteins. Heat shock proteins are molecular chaperones that are constitutively expressed in several eukaryotic cells and have been shown to protect against various stressors. However, the changes in the phosphorylation of atrophy-related heat shock protein 27 (HSP27) are still poorly understood in skeletal muscles. In this study, we examine whether or not phosphorylation of HSP27 is changed in the skeletal muscles after cast immobilization and serum-free starvation with low glucose in a time-dependent manner. [Methods] We undertook a HSP27 expression and high-resolution differential proteomic analysis in skeletal muscles. Furthermore, we used western blotting to examine protein expression and phosphorylation of HSP27 in atrophied gastrocnemius muscle strips and L6 myoblasts. [Results] Cast immobilization and starvation significantly upregulated the phosphorylation of HSP27 in a time-dependent manner, respectively. [Conclusion] Our results suggest that cast immobilization- and serum-free starvation-induced atrophy may be in part related to changes in the phosphorylation of HSP27 in rat skeletal muscles.
Key words: Heat shock protein 27, Cast immobilization and serum-free starvation, Muscle atrophy

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INTRODUCTION

Heat shock protein 27 (HSP27), a member of the widely distributed heat shock protein family, is an ubiquitous small heat shock protein also called a stress protein that is induced in response to stimulation such as hyperthermia, oxidative stress and nutritional deficiency[1–4]. Small heat shock proteins including HSP27 are characterized by low molecular weights (12–43 kDa) and share a conserved α–crystallin domain located at the carboxyl terminal[3, 4]. The HSP27 gene contains three exons encoding 205 amino acids and contains two functional binding sites for heat shock elements (HSEs)[4, 5]. In addition to its common roles, such as in chaperone functions, HSP27 can suppress apoptosis and oxidative stress[1, 6, 7]. Furthermore, HSP27 has been implicated in several diseases, such as atherosclerosis, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, renal fibrosis, and various nephropathies[5, 8, 9]. Meanwhile, muscle atrophy has proven to be a significant problem in the area of physical therapy[10–15]. However, the changes in the phosphorylation of HSP27 in atrophic conditions are not fully understood. Therefore, in the present study, we demonstrated the changes in the phosphorylation of HSP27 caused by external cast immobilization- and serum-free starvation-induced atrophy in skeletal muscles.

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MATERIALS AND METHODS

Male Sprague-Dawley rats (n=17) were anaesthetized during the attachment of the plaster of paris casting material[10, 11]. Two-dimensional gel electrophoresis and matrix-assisted laser desorption ionization time-of-flight/time-of-flight mass spectrometry were performed as reported in our previous studies[12]. L6 myoblasts were purchased from the American Type Culture Collection (Rockville, MD, USA) and cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and a high concentration of D-glucose (4,500 mg/L). The serum-free starvation group was grown to 60–70% confluence and undernourished in DMEM containing a low concentration of D-glucose (1,000 mg/L) without FBS for 3, 6, 12, 24, 48, and 72 h, respectively[10, 15]. Furthermore, to measure the phosphorylation of HSP27, gastrocnemius muscle strips were isolated after specific intervals of cast immobilization. The samples were then homogenized in a sample buffer. Proteins (35–50 μg/lane) were separated on 12% polyacrylamide sodium dodecyl sulfate gels and then transferred electrophoretically to a polyvinylidene fluoride membrane (Millipore; Bedford, MA, USA)[11]. Anti-HSP27 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody-specific bands were quantified using an image analyzer (Bio-Rad). The present investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The protocol for the study was approved by the Committee of Ethics in Research of the University of Yongin, in accordance with the terms of Resolution 5-1-20, December 2006. Data were expressed as means±SEM. The data were statistically evaluated using Student's t-tests for comparisons between pairs of groups and by ANOVA for multiple comparisons. A p value of < 0.05 was considered to be statistically significant.

RESULTS

The density of HSP27 spots detected in the gastrocnemius muscles is shown in Fig. 1A. The phosphorylation of HSP27 was significantly increased after 3, 7, 14, and 21 days of cast immobilization compared with the levels of phosphorylation in the control group (n=4, Fig. 1B, Table 1). Furthermore, serum-free starvation with the low concentration of glucose after 3, 6, 12, 24, 48, and 72 hours significantly increased the phosphorylation of HSP27, respectively (n=4; Fig. 1B, Table 1). However, the expression of HSP27 was not changed in the experimental group compared with that in the control group, respectively (n=4, Fig. 1B, Table 1).

DISCUSSION

It is well known that previous studies and our reports using a rat model of disuse atrophy induced by cast immobi-

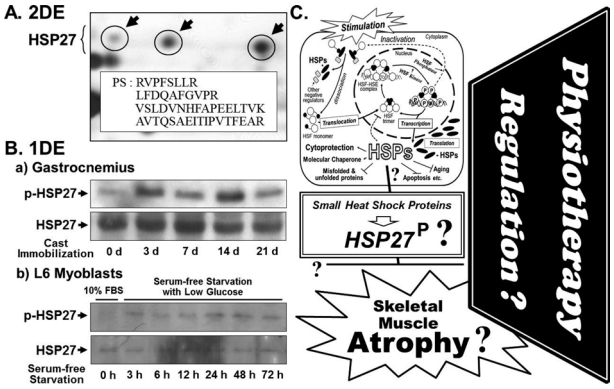

Fig. 1. Changes in phosphorylation of HSP27 and schematic representation of cellular response caused by cast immobilization and serum-free starvation with a low concentration of glucose in skeletal muscles
Proteomic (A) and immunoblotting (B) analysis in cast-immobilized skeletal muscle. 2DE and 1DE, two- and one-dimensional gel electrophoresis; HSP27, heat shock protein 27; PS, peptide sequence; R, arginine (Arg); V, valine (Val); P, proline (Pro); F, phenylalanine (Phe); S, serine (Ser); L, leucine (Leu); D, aspartic acid (Asp); Q, glutamine (Gln); A, alanine (Ala); G, glycine (Gly); N, asparagine (Asn); H, histidine (His); E, glutamic acid (Glu); T, threonine (Thr); K, lysine (Lys); I, isoleucine (Ile); FBS, fetal bovine serum; d, days; h, hours; p, phosphorylated protein; HSF, heat shock transcription factor; HSE, heat shock response element; HSPs, heat shock proteins (Kim et al.[1]).

Table 1. Changes in expression and phosphorylation of HSP27 of skeletal muscles during cast immobilization and serum-free starvation with a low concentration of glucose

Cast Immobilization	p-HSP27 (%)	HSP27 (%)	Serum-free Starvation with Low Glucose	p-HSP27 (%)	HSP27 (%)
0 day	100.0±0.0	100.0±0.0	0 hour	100.0±0.0	100.0±0.0
3 days	297.0±32.6*	108.3±6.6	3 hours	253.7±37.3*	92.7±7.1
7 days	193.7±31.5*	120.0±15.8	6 hours	137.0±8.5*	105.0±2.9
14 days	398.7±26.9*	101.7±6.1	12 hours	158.7±10.2*	105.3±3.2
21 days	179.3±25.7*	105.7±5.2	24 hours	304.7±53.2*	104.3±3.8
			48 hours	268.3±34.9*	94.3±3.4
			72 hours	175.7±15.6*	97.7±6.2

Means±SEM. p, phosphorylated protein; HSP27, heat shock protein 27. The basal levels of abundance and phosphorylation of HSP27 in controls (0 days and 0 hours) were considered to be 100%. *Versus the 0 day control, $p<0.05$.

lization indicate the loss of muscle mass and cross-sectional area due to a decrease in the rate of protein synthesis[10–14]. Often, skeletal muscle atrophy is a necessary phenomenon in clinical conditions such as long-term bed rest, sarcopenia, and articular fixation in the area of orthopedic physiotherapy[10–12, 16, 17]. The heat shock proteins consist of a number of highly conserved proteins thought to play protective roles, such as in molecular chaperone and antioxidant effects in cells subjected to high temperature, hypoxia, and other stresses[1, 4, 7]. In particular, HSP27 is involved in protection against programmed cell death by inhibition of caspase-dependent apoptosis[6, 18]. Phosphorylated HSP27 also prevents filament degeneration and promotes polymerization in the case of actin filament regulation[19–21]. In the present study, we demonstrated that immobilization- and starvation-induced atrophy increased the phosphorylation of HSP27 in a time-dependent manner. Based on these results, we cautiously speculate that the increment in HSP27 phosphorylation aids in adaptation to and prevention of stress induced by environmental stresses, such as cast immobilization and serum-free starvation with a low concentration of glucose. Furthermore, our previous data showed that the transcriptional regulation of MuRF-1 is upregulated in the development of both immobilization- and starvation-induced muscle atrophy[10]. Simultaneously, the mitogen-activated protein kinases such as extracellular signal-regulated kinase 1/2, stress-activated protein kinase/*c-Jun* NH_2-terminal kinase, and p38 mitogen-activated protein kinase are involved in muscle atrophy induced by immobilization and cell starvation[10, 11]. Our previous reports also demonstrated that cast immobilization increases the expression of myoglobin and decreases the phosphorylation of cofilin in skeletal muscle cells[13–15]. These reports have carefully suggested that the change in mitogen-activated protein kinases, myoglobin, and cofilin aids in adaptation to stress induced by cast immobilization and serum-free starvation with a low concentration of glucose. However, further systematic studies in the area of physiotherapy, such as with electro-, neuro-, hydro-, and thermotherapy, are needed to demonstrate the mechanism of HSP27 in muscle atrophic conditions[22–27] (Fig. 1C). In summary, phosphorylation of HSP27 was increased in cast-immobilized gastrocnemius muscles and in L6 myoblasts subjected to serum-free starvation, respectively. The present results suggest that stress-induced atrophy may be in part mediated by HSP27 from skeletal muscles.

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