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Umbilical cord mesenchymal stem cell-conditioned media prevent muscle atrophy by suppressing muscle atrophy-related proteins and ROS generation

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Abstract The therapeutic potential of mesenchymal stem cell-conditioned medium (MSC-CM) has been reported with various types of disease models. Here, we examine the therapeutic effect of umbilical cord MSC-CM (UCMSC-CM) on muscle-related disease, using a dexamethasone (Dex)-induced muscle atrophy in vitro model. The expressions of muscle atrophy-related proteins (MuRF-1 and MAFbx) and muscle-specific proteins (desmin and myogenin) were evaluated by Western blot analysis. The level of production of reactive oxygen species (ROS) was determined using a 2′,7′-dichlorofluorescein diacetate (DCFDA) dye assay. The expression of antioxidant enzymes (copper/zinc-superoxide dismutase (Cu/Zn-SOD), manganese superoxide dismutase (MnSOD), glutathione peroxidase-1 (GPx-1), and catalase (CAT)) was verified by reverse transcription polymerase chain reaction (RT-PCR). When L6 cells were exposed to Dex, the expression of muscle atrophy-related proteins was increased by 50–70%, and the expression of muscle-specific proteins was in turn decreased by 23–40%. Conversely, when the L6 cells were co-treated with UCMSC-CM and Dex, the expression of muscle atrophy-related proteins was reduced in a UCMSC-CM dose-dependent manner and the expression of muscle-specific proteins was restored to near-normal levels. Moreover, ROS generation was effectively suppressed and the expression of antioxidant enzymes was recovered to a normal degree. These data imply that UCMSC-CM clearly has the potential to prevent muscle atrophy. Thus, our present study offers fundamental data on the potential treatment of muscle-related disease using UCMSC-CM.

Keywords Umbilical cord mesenchymal stem cell (UCMSC) · Umbilical cord mesenchymal stem cell-conditioned medium (UCMSC-CM) · Muscle atrophy · Dexamethasone · L6 skeletal muscle cell

Chan-Mi Park and Mi Jin Kim contributed equally to this work.

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Introduction

Recent trends in the current population's prolonged life expectancy and reduced activities in modern-day lifestyle have led to an increase in the incidence of muscle atrophy. With this realization, the need for the development of treatments for this disease has been highlighted. Muscle atrophy is a process of muscle wasting along with the activation of muscle proteolytic systems. Many cases can be seen in patients with less movement, prolonged bed rest, and the casting of particular body parts (Senf *et al.* 2008). Also, abuse of steroids can lead to muscle atrophy and stimulate the production of myostatin,

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which takes part in the reduction of muscle protein synthesis (Schakman *et al.* 2013). Eventually, this gives rise to not only an increase in the imbalance of muscle mass, but also an increase in the generation of reactive oxygen species (ROS) within mitochondria, leading to an increase in apoptosis (Lee and McPherron 1999).

Current treatments for muscle atrophy are electrical excitation, regular exercise using the treadmill, and chemical treatment (Gibson *et al.* 1988; Kouidi *et al.* 1998). However, these regimens cannot be applied to the symptoms of bed-ridden patients. Thus, a more effective solution needs to be developed in order to cover broader causations. Recently, many researchers have attempted to develop therapeutic modalities using stem cells (Gnecchi *et al.* 2006; Chen *et al.* 2008; Choi and Park 2014; Kim *et al.* 2015). In particular, both embryonic and adult stem cells have been an effective treatment for muscular diseases, including previously incurable disease (Jackson *et al.* 2001; Yohn *et al.* 2008; Corti *et al.* 2010; Stecklum *et al.* 2015). For example, stem cell therapy for amyotrophic lateral sclerosis, also known as Lou Gehrig's disease, has been reported (Kim *et al.* 2014a).

The therapeutic mechanisms of implanted mesenchymal stem cell (MSC) can be explained by the fact that various factors are secreted into the conditioned medium (CM), such as growth factors, cytokines, extracellular matrices, and antioxidant proteins (Primeau *et al.* 2002; Kern *et al.* 2006; Ranganath *et al.* 2012). These factors are able to promote antioxidant, immunosuppressive, anti-apoptotic, and cell proliferative effects at a transplanted site (Klopp *et al.* 2011). This hypothesis has been supported by various CM-related studies. For instances, Moghadasali et al. showed that bone marrow-derived mesenchymal stem cell-conditioned medium (MSC-CM) was able to regenerate a damaged proximal tubule (Moghadasali *et al.* 2013). In addition, Cantinieaux et al. demonstrated that possible MSC-CM-only treatments are efficacious, by showing improved brain function of a spinal cord-impaired mouse through the injection of CM (Cantinieaux *et al.* 2013). However, studies of the therapeutic effect of MSC-CM on muscle atrophy have rarely been reported both in vitro and in vivo.

In this study, we attempt to examine the therapeutic effect of umbilical cord (UC) MSC-CM (UCMSC-CM) on dexamethasone (Dex)-induced muscle atrophy in L6 skeletal muscle cells. We investigated the effect of UCMSC-CM on changes in the expression of muscle atrophy-related proteins (MuRF-1 and MAFbx) and muscle-specific proteins (desmin and myogenin). In addition, the protective effect of UCMSC-CM on the atrophied muscle cells was assessed by comparing the changes in ROS generation and expression of antioxidant enzymes.

Materials and Methods

Umbilical cord mesenchymal stem cell preparation. This study was performed with the approval of an Institutional Review Board from the CHA University (IRB No.: 201412-BR-003-02; Seongnam, Korea). The protocols for human UCMSC isolation and culture have been described previously (Kim *et al.* 2013b). Briefly, healthy volunteers at the CHA General Hospital (Seoul, Korea) donated human UC samples, and the samples were used within 24 h. To isolate the UCMSC, Wharton's jelly was sliced into 5-mm explants after removing the umbilical vessels, and the slices were subsequently attached and cultured in minimum essential medium Eagle alpha modification (α-MEM; Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (FBS; Hyclone), 100 IU/mL penicillin, and 100 mg/mL streptomycin (P/S; Hyclone) on T-175 flasks (SPL Life Sciences Co., Pocheon, Korea). The medium was renewed every 3 d, and we observed UCMSC populations appearing as outgrowths from the UC fragments on day 6 of culture. After 15 d of culture, the adherent spindle-shape cells were passaged when the confluence reached the predetermined level (80–90%). Cells were maintained at 37°C in a humidified incubator containing 5% CO_2. UCMSCs at passage 4 or 5 were used in all experiments.

Collection of umbilical cord mesenchymal stem cell-conditioned medium. UCMSC was seeded in T-175 flasks (SPL) at a density of 2×10^6 cells in α-MEM, with 100 IU/mL penicillin and 100 mg/mL streptomycin (P/S), and 10% FBS for the MSC growth. The cultures were maintained in a humidified incubator with 5% CO_2 at 37°C, and the culture medium was changed every 2 d. When the cells reached roughly 80–90% confluence, the culture medium was removed and attached cells on the flasks were washed twice with Dulbecco's phosphate-buffered saline (DPBS; Hyclone). Then, serum-free α-MEM supplemented with 1% P/S was added. After 48 h of culture in a humidified incubator with 5% CO_2 at 37°C, the medium was centrifuged at 13,000 rpm for 15 min to collect UCMSC-CM. The CM was then lyophilized using a freeze dryer (FDU-8612, Operon Co., Gimpo, Korea). It was stored at −20°C until further use. Muscle atrophy-induced L6 skeletal muscle cells were treated with different concentrations of UCMSC-CM (1, 5, and 10 μg/mL).

L6 muscle cell culture. L6 rat skeletal muscle cells were purchased from the American Type Culture Collection (CRL1458, ATCC, Manassas, VA). They were cultured in Dulbecco's modified Eagle medium (DMEM; Hyclone) supplemented with 10% FBS and 1% P/S. The cells at passage 5 were plated in T-175 flasks (SPL) at a density of 1×10^6 cells. When the cells were approximately 80% confluent, they were seeded in six-well culture plates (SPL; 2×10^5 cells/well).

Cells were treated with Dex (1 μM) as an in vitro model for muscle atrophy.

Western blotting. After treatment with Dex and UCMSC-CM, cells were lysed with RIPA buffer containing a protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Total extracted protein concentration was evaluated by an ELISA microplate reader at 595 nm. The proteins were immediately denatured using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (62.5 mM Tris–HCl pH 6.8, 2% SDS, 10% glycerol, and 5% 2-mercaptoethanol) and heated for 5 min at 100°C. The protein samples were separated by 10% SDS-PAGE. Separated proteins were transferred to a nitrocellulose membrane (Whatman, Dassel, Germany) for 3 h at 4°C. Subsequently, the membrane was blocked with 3% bovine serum albumin (BSA; Bio Basic Inc., Ontario, Canada) with 0.1% Tween-20 in PBS at room temperature. The membrane was incubated overnight with each primary antibody at 4°C. Western blotting was carried out using anti-desmin, 1:1000 dilution (Abcam Inc., Cambridge, MA; ab16200); anti-myogenin, 1:1000 dilution (Santa Cruz Biotechnology Inc., Santa Cruz, CA; sc-12732); anti-β-actin, 1:5000 dilution (Santa Cruz Biotechnology Inc.; sc-47778); anti-muscle ring-finger protein-1 (MuRF-1), 1:1000 dilution (Santa Cruz Biotechnology Inc.; sc-27642); and anti-muscle atrophy F-box (MAFbx), 1:1000 dilution (Santa Cruz Biotechnology Inc.; sc-27645). After washing with PBS-T, the blots were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies corresponding to each primary antibody; goat anti-rabbit, 1:5000 dilution (Santa Cruz Biotechnology Inc.; sc-2004), goat anti-mouse, 1:5000 dilution (Santa Cruz Biotechnology Inc.; sc-2005); and donkey anti-goat, 1:5000 dilution (Santa Cruz Biotechnology Inc.; sc-2020). Target proteins were visualized using enhanced chemiluminescence detection (ECL component from Pierce Clarity™ and Western ECL Substrate from Bio-Rad Laboratories, Hercules, CA) and detected using LAS-4000 (Fuji Photo Film Co., Tokyo, Japan).

Reverse transcription polymerase chain reaction. Total RNA from the L6 cells was isolated using the easy-spin™ total RNA extraction kit (iNtRON Biotechnology Inc., Seoul, Korea) according to the manufacturer's instructions. cDNA synthesis was carried out from 1 μg of pure total RNA using the Reverse Transcription 5× Master Premix (ELPIS Biotech Inc., Daejeon, Korea), followed by PCR amplification. The amplification was conducted under the following conditions: predenaturation at 95°C for 5 min; 27 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. PCR product was visualized using electrophoresis on a 1.5% (*w/v*) agarose gel stained with ethidium bromide. The image of the gel was analyzed using a ChemiDoc™ XRS+ System (Bio-Rad). Primer sequences are listed in Table 1.

Measurement of superoxide dismutase activity. Superoxide dismutase (SOD) activity in UCMSC-CM was measured by the SOD Assay kit (Sigma-Aldrich, Chemie GmbH, Germany). The activity was determined following the manufacturer's instructions. Twenty microliters of sample solution was added to 200-μL WST working solution. The mixture was incubated in a 96-well plate at 37°C in a 5% CO_2 for 20 min after gentle shaking. Subsequently, 20-μL enzyme working solution was added. The absorbance of the mixture was spectrophotometrically measured at 450 nm using an ELISA microplate reader (Epoch Spectrometer, BioTek Inc., Winooski, VT), and the SOD activity was calculated using the following Eq. (1):

$$\%\ \text{SOD activity} = \frac{[(\text{Blank}_1\text{-Blank}_3)\text{-}(\text{Sample-Blank}_2)]}{(\text{Blank}_1\text{-Blank}_3)} \times 100 \quad (1)$$

where blank 1 was a mixture of the 200-μL working solution, 20-μL enzyme working solution, and 20 μL ddH_2O; blank 2 contained the 20-μL sample solution with 200-μL working solution and 20 μL dilution buffer; blank 3 was a mixture of the 20 μL ddH_2O, 200-μL working solution, and 20 μL dilution buffer.

Measurement of reactive oxygen species. Measurement of ROS generation in L6 cells was accessed by the 2′,7′-dichlorofluorescein diacetate (DCFDA) assay (Life Technologies Inc., Gaithersburg, MD). For the measurement of ROS generation, the cells were seeded at 1×10^5 cells/well in a 48-well plate and cultured for 48 h in DMEM with 10% FBS and 1% P/S. When the cells reached the determined confluence (80–90%), they were washed once with HBSS (Gibco Laboratories, Grand Island, NY) and treated with 1, 5, or 10 μg/mL UCMSC-CM for 2 h. Subsequently, the cells were incubated at 37°C in 5% CO_2 for 30 min, following treatment with 2 μM DCFDA. Relative fluorescence of the samples was measured using a Synergy Mx Multi-Mode Reader (BioTek Inc.) at excitation and emission wavelengths of 485 and 528 nm, respectively. Data were obtained from three independent measurements and expressed as the mean standard deviation.

Statistical analysis. All in vitro experiments were independently performed at least three times. Data were analyzed using the SigmaPlot 12.0 software (Systat Software, San Jose, CA). When comparing more than two groups, one-way analysis of variance (ANOVA) combined with the Bonferroni post hoc test was used. For comparison of two samples, a Student's *t* test was used. Quantitative results are shown as the mean± standard deviation for each experiment. $P<0.05$ was considered statistically significant.

Table 1. Primer sequences used in this study

Primers	Sequences	
	Forward	Reverse
Cu/Zn-SOD	CGTCATTCACTTCGAGCAGA	CAGGTCTCCAACATGCCTCT
MnSOD	GGCCAAGGGAGATGTTACAA	TGCAGTGGGTCCTGATTAGA
Gpx-1	TGAGAAGTGCGAGGTGAATG	GTATCTGCGCACTGGAACAC
CAT	ATCAGGGATGCCATGTTGTT	TGCAAGTCTTCCTGCCTCTT
GAPDH	AAGGTCATCCCAGAGCTGAA	AGGAGACAACCTGGTCCTCA

Results

Umbilical cord mesenchymal stem cell-conditioned medium inhibits expression of muscle atrophy-related proteins in muscle atrophied cells. Firstly, we selected a statistically significant concentration of 1 μM Dex as an effective concentration to induce muscle atrophy in L6 cells by considering cell viability and the expression of muscle atrophy-related proteins (Supplementary Fig. 1). Secondly, in order to investigate changes in muscle atrophy-related proteins (MuRF-1 and MAFbx), UCMSC-CM was added at a concentration of 1, 5, and 10 μg/mL along with Dex. As shown in Fig. 1, treatment with 1 μg/mL of CM did not showed any significant effect on expression of MuRF-1 and MAFbx, but treatments with Dex at above 5 μg/mL significantly suppressed the expression level of those proteins compared with only Dex-treated group. Conversely, the treatment with UCMSC-CM dose-dependently decreased the expression of both muscle atrophy-related proteins compared with Dex-treated group. These results imply that UCMSC-CM has the potential to inhibit expression of the muscle atrophy-related proteins, MuRF-1 and MAFbx.

Umbilical cord mesenchymal stem cell-conditioned medium enhances expression of muscle-specific proteins in muscle atrophied cells. We observed changes in the expression of muscle-specific proteins (desmin and myogenin) in L6 cells upon treatment with Dex. As shown in Fig. 2, expression of both proteins was decreased compared with the untreated control. Conversely, UCMSC-CM treatment at both 5 and 10 μg/mL remarkably improved expression of those proteins compared with the muscle atrophy-induced L6 cells (Dex-treated group). These observations indicate that UCMSC-CM has the potential to enhance expression of the muscle-specific proteins, desmin and myogenin.

Umbilical cord mesenchymal stem cell-conditioned medium suppresses muscle atrophy-induced reactive oxygen species generation in muscle atrophied cells. It is well known that ROS plays an important role in inducing muscle atrophy. Therefore, we measured SOD activity of UCMSC-CM (ranging from 1 to 50 μg/mL). SOD activity of UCMSC-CM was

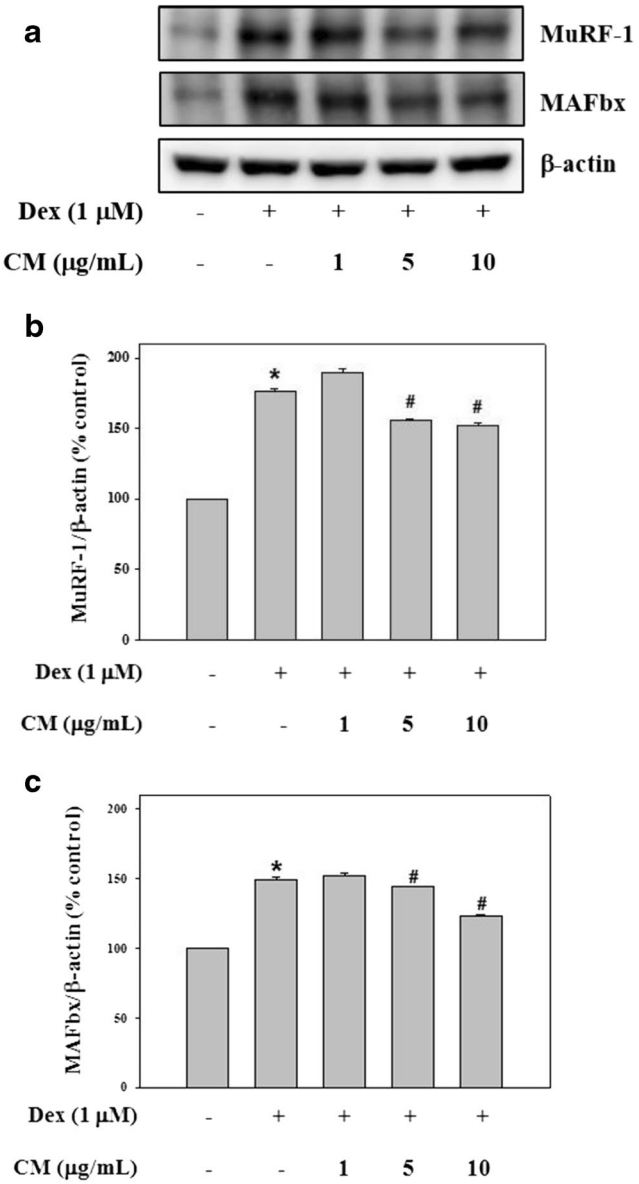


Figure 1. The effect of UCMSC-CM on muscle atrophy-related proteins in muscle atrophied cells. Cells were treated with CM (1–10 μg/mL) and Dex (1 μM) after 24 h of stimulation. A representative Western blot (*a*) and quantitation analysis (*b*, *c*) are shown (*$P<0.05$ vs control, $^{\#}P<0.05$ vs Dex).

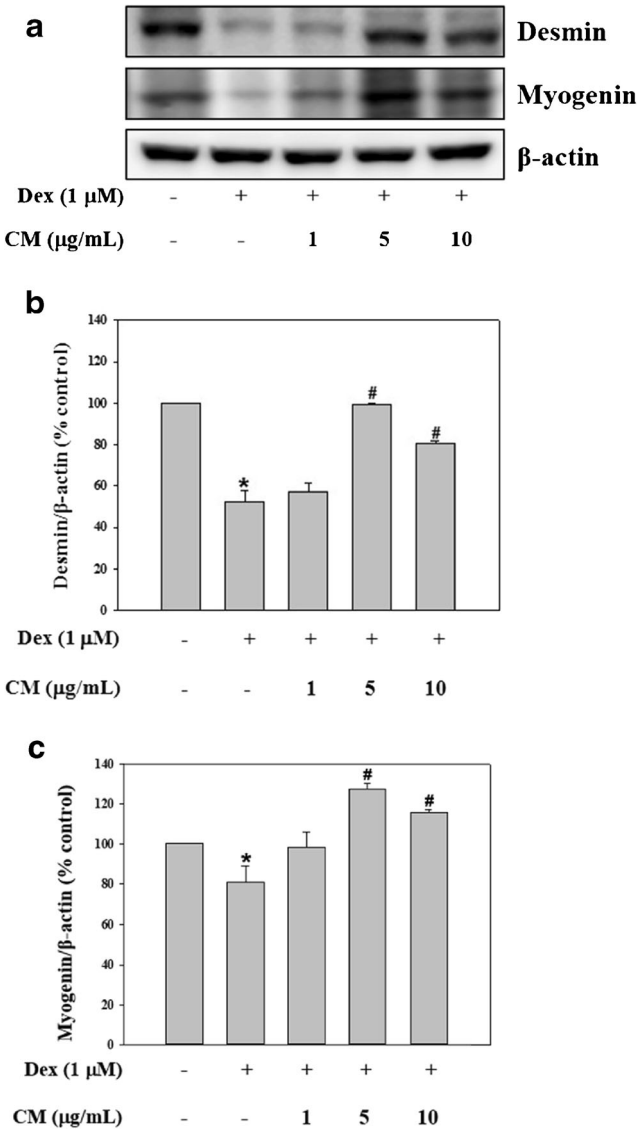


Figure 2. The effect of UCMSC-CM on muscle-specific proteins in muscle atrophied cells. Cells were treated with CM (1–10 μg/mL) and Dex (1 μM) at 24 h. Protein levels of desmin and myogenin were determined by Western blotting (*a*) and normalized with β-actin (*b*, *c*) (**P*<0.05 vs control, $^{\#}P$<0.05 vs Dex).

dose-dependently increased from 20 to 50% compared with the control (Fig. 3; P<0.05). In addition, a DCFDA assay was carried out to monitor patterns of ROS generation by Dex and UCMSC-CM in L6 cells (Fig. 4). We found that treatment with Dex sharply increased the DCFDA intensity to 70% within 5 min compared with the control. Following this, the DCFDA intensity was slowly decreased for a period of 2 h. Contrary to the Dex-treated group, co-treatment of UCMSC-CM with Dex showed a significant suppression of ROS generation in L6 cells with all tested UCMSC-CM concentrations (ranging from 1 to 10 μg/mL). This result indicates that excessive ROS generation within the cells treated with Dex can be effectively suppressed by the addition of UCMSC-CM.

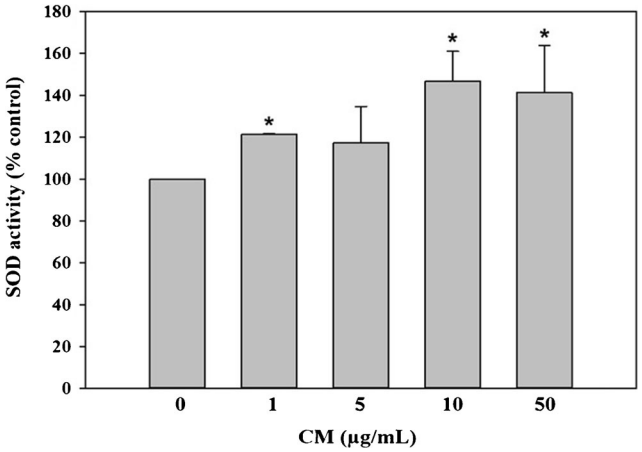


Figure 3. Changes in the SOD activity in UCMSC-CM. The level of SOD activity in UCMSC-CM was measured using a SOD assay kit (**P*<0.05 vs control).

Umbilical cord mesenchymal stem cell-conditioned medium improves expression of antioxidant enzymes in muscle atrophied cells. To study a potential protective mechanism of UCMSC-CM on Dex-induced oxidative-damaged cells, messenger RNA (mRNA) expression of the antioxidant enzymes copper/zinc-superoxide dismutase (Cu/Zn-SOD), manganese superoxide dismutase (MnSOD), glutathione peroxidase-1 (GPx-1), and catalase (CAT) was analyzed by reverse transcription polymerase chain reaction (RT-PCR). As shown in Fig. 5, mRNA expression of all these enzymes was drastically reduced by 30–60% by treatment of Dex, compared with the control group (P<0.05). Conversely, co-treatment of UCMSC-CM at 1, 5, and 10 μg/mL, with Dex, dose-dependently upregulated mRNA expression of all these enzymes except for the Cu/Zn-SOD, which was significantly upregulated, but not shown CM dose-dependent increases. Most notably, among the examined antioxidant enzymes, GPx-1 is influenced the greatest by Dex. In contrast, upon treatment with UCMSC-CM at 5 and 10 μg/mL, GPx-1 was the enzyme that was most increased above the level seen in the control group. In addition, treatment with UCMSC-CM at 10 μg/mL not only showed full recovery of mRNA expression similar to that of the control, but also this protective potential was comparable to 0.2 mM vitamin C.

Discussion

Many studies have reported that various factors secreted from MSC, such as cytokines and growth factors, are able to stimulate cell differentiation as well as to promote therapeutic functions (Gnecchi *et al.* 2006; Chen *et al.* 2008; Kim *et al.* 2014b). These factors are accumulated within UCMSC-CM, and implantation of UCMSC-CM can be considered as a novel approach to tissue engineering and regenerative medicine. Furthermore, this technique may be attractive as an alternative

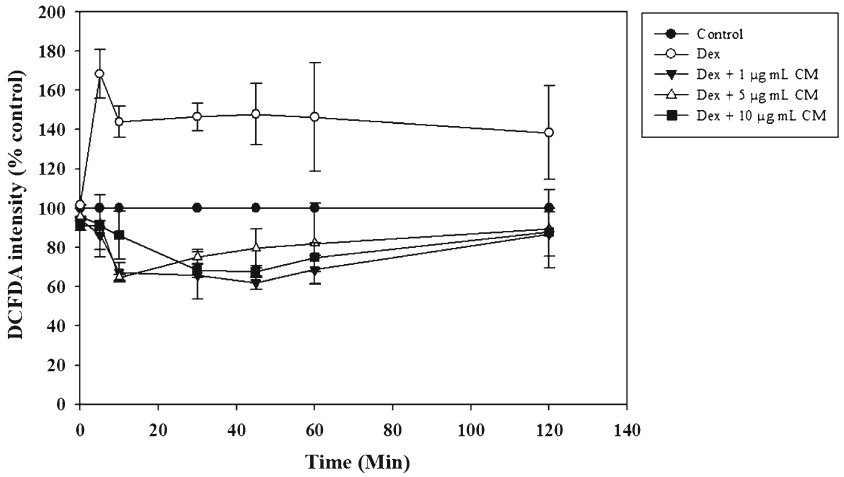


Figure 4. Effect of UCMSC-CM on ROS generation in muscle atrophied cells. Cells were treated with CM (1–10 μg/mL) and Dex (1 μM) for 2 h. ROS generation was measured by the DCFDA assay.

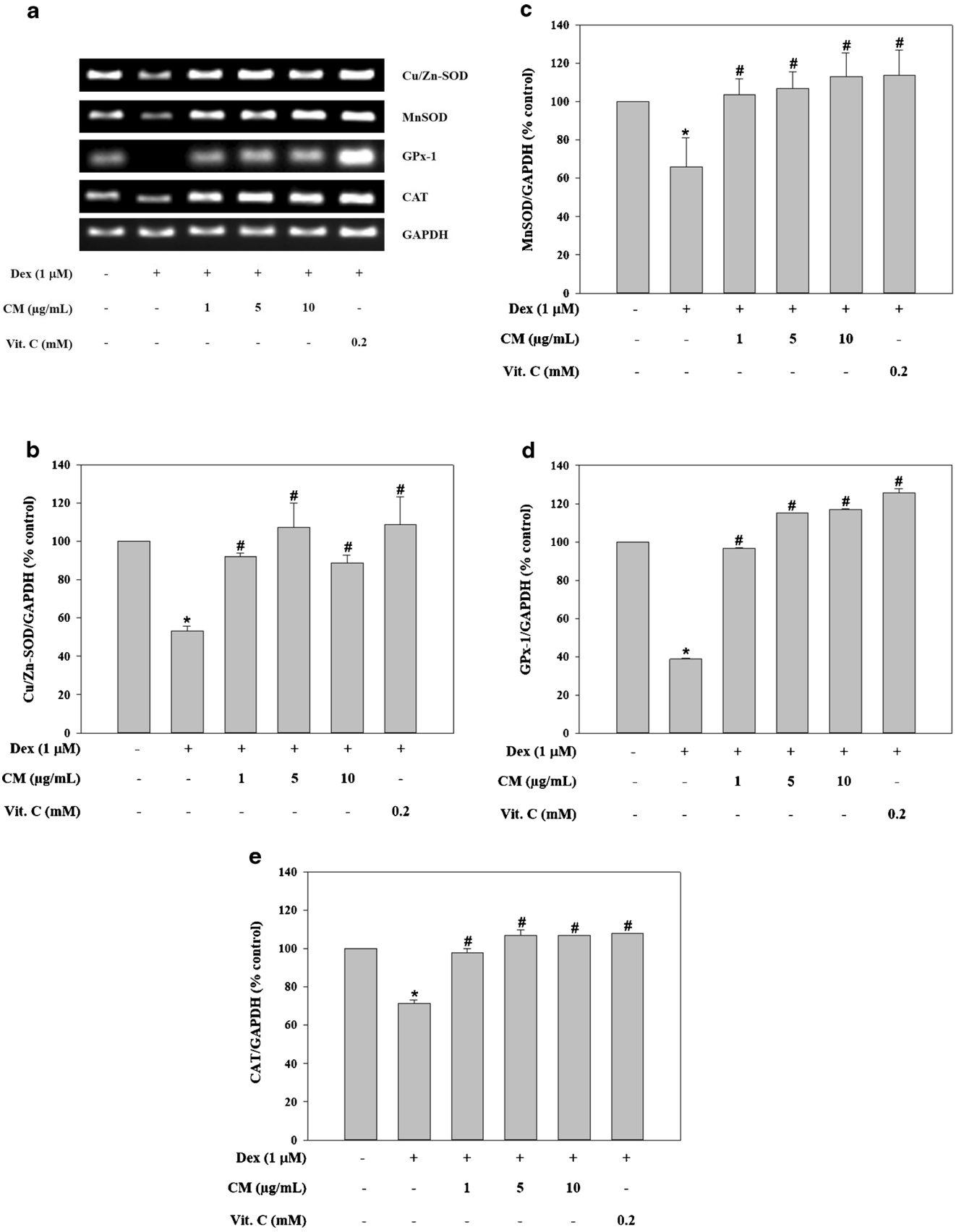
to direct implantation of MSC, due to its relative low cost and simple handing process. In addition, previous studies provide much evidence that CM has beneficial effects in the correction of various disease types (Angoulvant *et al.* 2011; Kim *et al.* 2012; Moghadasali *et al.* 2013). However, a therapeutic effect of MSC-CM on muscle atrophy has rarely been reported. In the present study, we attempted to examine the protective effect of UCMSC-CM on muscle atrophy using Dex-induced muscle atrophied L6 skeletal muscle cells. To this end, we investigated the effect of UCMSC-CM on expression patterns of muscle-related proteins and ROS generation.

We observed a significant downregulation of muscle atrophy-related protein expression and an increase in the expression of muscle-specific proteins when various concentrations of UCMSC-CM were co-treated with Dex in muscle atrophied L6 skeletal muscle cells (Figs. 1 and 2). From previous studies, it is known that abuse of steroids can stimulate the expression of proteolytic factors such as MAFbx and MuRF-1 within myoblasts. Eventually, the increased factors can result in muscle atrophy (Sandri *et al.* 2004; Bonaldo and Sandri 2013). In addition, desmin and myogenin, which are muscle-specific proteins, were known to be important in muscle cell proliferation and differentiation of satellite cells into myoblasts (Rantanen *et al.* 1995). This has been employed as a marker of regeneration in muscle fibers. Therefore, these results show that UCMSC-CM plays a protective role in muscle atrophied cells via the prevention of the muscle-specific protein loss as well as the inhibition of the level of muscle atrophy-related proteins. UCMSC-CM has the potential to prevent muscle atrophy.

Muscle atrophy not only causes an increase in the imbalance of muscle mass, but also causes an increase in ROS generation in mitochondria (Lee and McPherron 1999). ROS can lead to mitochondrial release of cytochrome c, resulting in the activation of caspase, which is an apoptosis-related endoprotease that degrades proteins and causes programmed cell death (Leeuwenburgh 2003). This evidence suggests that ROS may play an important role during muscle protein degradation in the mechanism of muscle atrophy. As shown in Fig. 3, UCMSC-CM has a potential ability to block the oxidative stress in muscle atrophied cells. Also, we found that UCMSC-CM was able to inhibit Dex-induced ROS generation by observing the DCFDA intensity in the muscle atrophied cells (Fig. 4). Thus, UCMSC-CM clearly demonstrates that it is able to protect cells from apoptosis, which occurs following ROS generation in the mechanism of muscle atrophy.

In the antioxidant enzyme system, SODs, which include Cu/Zn-SOD and MnSOD, catalyze the dismutation of superoxide radicals into H_2O_2, which is further metabolized to H_2O and O_2 by CAT and GPx-1 (Hussain *et al.* 2004). Previous studies, in addition to our present study, have found that the level of antioxidant enzymes was significantly reduced in both in vivo and in vitro models of muscle atrophy (Kim *et al.* 2012, 2015). In contrast, we confirmed that treatment with UCMSC-CM strongly increased mRNA levels of antioxidant enzymes in muscle atrophied cells (Fig. 5). Also, considering significant changes in mRNA expression of GPx-1, which is a major isoform that is active against H_2O_2, it can be seen that the level of GPx-1 was mostly affected by the level of oxidative stress (Li *et al.* 2000). These results indicate that UCMSC-CM leads to downregulation of oxidative stress and upregulation of those enzymes that may be beneficial to the protection of muscle mass and the reduction of oxidative stress.

It is widely known that many therapeutic factors are secreted by MSCs into CM such as growth factors, cytokines, chemokines, extracellular matrix (ECM), and hormones (Ranganath *et al.* 2012). In particular, hepatocyte growth factor (HGF); granulocyte–macrophage colony-stimulating factor (GM-CSF); interleukins (IL) 6, 7, 8, and 11; tumor necrosis factor alpha (TNF-α); and vascular endothelial growth factor (VEGF) were found in MSC-CM (Friedman *et al.* 2007). These findings imply that CM may have promising


a
Cu/Zn-SOD
MnSOD
GPx-1
CAT
GAPDH
Dex (1 μM) - + + + + +
CM (μg/mL) - - 1 5 10 -
Vit. C (mM) - - - - - 0.2
b
Cu/Zn-SOD/GAPDH (% control)
0 20 40 60 80 100 120 140
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Dex (1 μM) - + + + + +
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Dex (1 μM) - + + + + +
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Vit. C (mM) - - - - - 0.2

◀ **Figure 5.** Effect of UCMSC-CM on antioxidant enzymes in muscle atrophied cells. Cells were treated with CM (1–10 μg/mL) and Dex (1 μM) after 24 h of stimulation. Expression level of Cu/Zn-SOD, MnSOD, CAT, and GPx-1 on 1 μM Dex and various concentrations of UCMSC-CM used to treat muscle atrophied cells. A representative western blot (*a*) and quantitation analysis (*b–e*) are shown (*$P<0.05$ vs control, $^{\#}P<0.05$ vs Dex).

possibilities in regenerative medicine involved with the immune system, central nervous system, blood vessels, and heart (Kim *et al.* 2013a, b). Especially, insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF), and interleukins-15 (IL-15) are known to affect muscle activity (Hamrick 2012). Moreover, in a previous study, transforming growth factor beta (TGF-β) was found to be important in cell growth, proliferation, and differentiation (Kollias and McDermott 2008). In addition, IGF, HGF, and IL-6 are able to quench ROS in tissues or organs (Kim *et al.* 2008). Therefore, those factors can be thought to affect the expression of muscle proteins and oxidative stress during muscle atrophy. Our study provides supporting evidence of such a paracrine effect of UCMSC and demonstrates that UCMSC-CM accelerates recovery from muscle atrophy.

In conclusion, the results show that UCMSC-CM has a very high potential to suppress the proteolytic system and ROS generation in muscle atrophied cells. Herein, the current study is a novel report of the use of UCMSC-CM for the improvement of muscle atrophy in vitro and may lead to the generation of therapeutic agents useful in the management of muscle diseases. However, there are still uncertainties regarding the paracrine effect of MSC, clinical optimization, and standard of manufacturing technique of CM. Thus, further studies are required in order to solve such problems and to understand the exact role that UCMSC-CM plays during muscle atrophy through the investigation of specific cytokines and growth factors excreted by MSC.

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References

Angoulvant D, Ivanes F, Ferrera R, Matthews PG, Nataf S, Ovize M (2011) Mesenchymal stem cell conditioned media attenuates in vitro and ex vivo myocardial reperfusion injury. J Heart Lung Transplant 30:95–102

Bonaldo P, Sandri M (2013) Cellular and molecular mechanisms of muscle atrophy. Dis Model Mech 6:25–39

Cantinieaux D, Quertainmont R, Blacher S, Rossi L, Wanet T, Noël A, Brook G, Schoenen J, Franzen R (2013) Conditioned medium from bone marrow-derived mesenchymal stem cells improves recovery after spinal cord injury in rats: an original strategy to avoid cell transplantation. PLoS One 8, e69515

Chen L, Tredget EE, Wu PY, Wu Y (2008) Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS One 3, e1886

Choi MY, Park KH (2014) Establishment of endogenous human tympanic membrane-derived somatic stem cells for stem cell therapy. In Vitro Cell Dev Biol Anim 50(8):747–755

Corti S, Nizzardo M, Nardini M, Donadoni C, Salani S, Ronchi D, Simone C, Falcone M, Papadimitriou D, Locatelli F (2010) Embryonic stem cell-derived neural stem cells improve spinal muscular atrophy phenotype in mice. Brain 133:465–481

Friedman R, Betancur M, Boissel L, Tuncer H, Cetrulo C, Klingemann H (2007) Umbilical cord mesenchymal stem cells: adjuvants for human cell transplantation. Biol Blood Marrow Transplant 13:1477–1486

Gibson J, Smith K, Rennie M (1988) Prevention of disuse muscle atrophy by means of electrical stimulation: maintenance of protein synthesis. Lancet 332:767–770

Gnecchi M, He H, Noiseux N, Liang OD, Zhang L, Morello F, Mu H, Melo LG, Pratt RE, Ingwall JS (2006) Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J 20:661–669

Hamrick MW (2012) The skeletal muscle secretome: an emerging player in muscle-bone crosstalk. BoneKey Rep 1(60):1–5

Hussain SP, Amstad P, He P, Robles A, Lupold S, Kaneko I, Ichimiya M, Sengupta S, Mechanic L, Okamura S (2004) p53-induced up-regulation of MnSOD and GPx but not catalase increases oxidative stress and apoptosis. Cancer Res 64:2350–2356

Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK, Goodell MA (2001) Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 107:1395–1402

Kern S, Eichler H, Stoeve J, Klüter H, Bieback K (2006) Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 24:1294–1301

Kim HO, Choi S-M, Kim H-S (2013a) Mesenchymal stem cell-derived secretome and microvesicles as a cell-free therapeutics for neurodegenerative disorders. Tissue Eng Regen Med 10:93–101

Kim HY, Kim H, Oh KW, Oh SI, Koh SH, Baik W, Noh MY, Kim KS, Kim SH (2014a) Biological markers of mesenchymal stromal cells as predictors of response to autologous stem cell transplantation in patients with amyotrophic lateral sclerosis: an investigator-initiated trial and in vivo study. Stem Cells 32:2724–2731

Kim J, Lee JH, Yeo SM, Chung HM, Chae JI (2014b) Stem cell recruitment factors secreted from cord blood-derived stem cells that are not secreted from mature endothelial cells enhance wound healing. In Vitro Cell Dev Biol Anim 50(2):146–154

Kim MJ, Kim YM, Kim Z-H, Heo S-H, Kim S-M, Hwang J-W, Chang W-J, Baek MJ, Choi YS (2015) Mesenchymal stem cells suppress muscle atrophy induced by hindlimb suspension. J Stem Cell Res Ther 5:2

Kim S-M, Moon SH, Lee Y, Kim GJ, Chung HM, Choi Y-S (2013b) Alternative xeno-free biomaterials derived from human umbilical cord for the self-renewal ex-vivo expansion of mesenchymal stem cells. Stem Cells Dev 22:3025–3038

Kim S-Y, Lee J-H, Kim HJ, Park MK, Huh JW, Ro JY, Oh Y-M, Lee S-D, Lee Y-S (2012) Mesenchymal stem cell-conditioned media recovers lung fibroblasts from cigarette smoke-induced damage. Am J Physiol Lung Cell Mol Physiol 302:L891–L908

Kim W-S, Park B-S, Kim H-K, Park J-S, Kim K-J, Choi J-S, Chung S-J, Kim D-D, Sung J-H (2008) Evidence supporting antioxidant action of adipose-derived stem cells: protection of human dermal fibroblasts from oxidative stress. J Dermatol Sci 49:133–142

Klopp AH, Gupta A, Spaeth E, Andreeff M, Marini F (2011) Concise review: dissecting a discrepancy in the literature: do mesenchymal stem cells support or suppress tumor growth? Stem Cells 29:11–19

Kollias HD, McDermott JC (2008) Transforming growth factor-β and myostatin signaling in skeletal muscle. J Appl Physiol 104:579–587

Kouidi E, Albani M, Natsis K, Megalopoulos A, Gigis P, Guiba-Tziampiri O, Tourkantonis A, Deligiannis A (1998) The effects of exercise training on muscle atrophy in haemodialysis patients. Nephrol Dial Transplant 13:685–699

Lee S-J, McPherron AC (1999) Myostatin and the control of skeletal muscle mass: commentary. Curr Opin Genet Dev 9:604–607

Leeuwenburgh C (2003) Role of apoptosis in sarcopenia. J Gerontol A Biol Sci Med Sci 58:999–1001

Li S, Yan T, Yang J-Q, Oberley TD, Oberley LW (2000) The role of cellular glutathione peroxidase redox regulation in the suppression of tumor cell growth by manganese superoxide dismutase. Cancer Res 60:3927–3939

Moghadasali R, Mutsaers HA, Azarnia M, Aghdami N, Baharvand H, Torensma R, Wilmer MJ, Masereeuw R (2013) Mesenchymal stem cell-conditioned medium accelerates regeneration of human renal proximal tubule epithelial cells after gentamicin toxicity. Exp Toxicol Pathol 65:595–600

Primeau AJ, Adhihetty PJ, Hood DA (2002) Apoptosis in heart and skeletal muscle. Can J Appl Physiol 27:349–395

Ranganath SH, Levy O, Inamdar MS, Karp JM (2012) Harnessing the mesenchymal stem cell secretome for the treatment of cardiovascular disease. Cell Stem Cell 10:244–258

Rantanen J, Hurme T, Lukka R, Heino J, Kalimo H (1995) Satellite cell proliferation and the expression of myogenin and desmin in regenerating skeletal muscle: evidence for two different populations of satellite cells. Lab Invest 72:341–347

Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, Picard A, Walsh K, Schiaffino S, Lecker SH, Goldberg AL (2004) Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. Cell 117:399–412

Schakman O, Kalista S, Barbe C, Loumaye A, Thissen J (2013) Glucocorticoid-induced skeletal muscle atrophy. Int J Biochem Cell Biol 45:2163–2172

Senf SM, Dodd SL, McClung JM, Judge AR (2008) Hsp70 overexpression inhibits NF-κB and Foxo3a transcriptional activities and prevents skeletal muscle atrophy. FASEB J 22:3836–3845

Stecklum M, Wulf-Goldenberg A, Purfürst B, Siegert A, Keil M, Eckert K, Fichtner I (2015) Cell differentiation mediated by co-culture of human umbilical cord blood stem cells with murine hepatic cells. In Vitro Cell Dev Biol Anim 51(2):183–191

Yohn DC, Miles GB, Rafuse VF, Brownstone RM (2008) Transplanted mouse embryonic stem-cell-derived motoneurons form functional motor units and reduce muscle atrophy. J Neurosci 28:12409–12418