수의학석사학위논문

동결해동 후 개의 헴옥시게네이즈-1 유전자 과발현 세포와 일반 지방유래 줄기세포 생존성 및 항산화능 비교연구

Comparison of Viability and Antioxidant Capacity after Freeze-Thawing Between Canine Adipose-Derived Mesenchymal Stem Cells and Heme Oxygenase-1-Overexpressed Cells

2015년 8월

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수의학과 임상수의학 전공

김 미 정

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김 미 정

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Abstract

Comparison of Viability and Antioxidant Capacity after Freeze-Thawing Between Canine Adipose-Derived Mesenchymal Stem Cells and Heme Oxygenase-1-Overexpressed Cells

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Allogenic adipose-derived mesenchymal stem cells (Ad-MSCs) are an alternative source for cytotherapy owing to their antioxidant and anti-inflammatory effects. Frozen-

thawed allogenic Ad-MSCs can be used instantly for this purpose. However, the viability and function of frozen-thawed Ad-MSCs have not been clearly evaluated. The purpose of this study was to compare the viability and function of Ad-MSCs and heme oxygenase-1 (HO-1)-overexpressed Ad-MSCs in vitro after freeze-thawing. The viability, proliferation, antioxidant capacity, and mRNA gene expression of growth factors were evaluated. Frozen-thawed cells showed significantly lower viability than fresh cells (77% for Ad-MSCs and 71% for HO-1 Ad-MSCs, $P < 0.01$). However, the proliferation rate of frozen-thawed Ad-MSCs increased, and did not differ from that of fresh Ad-MSCs after 3 days of culture. In contrast, the proliferation rate of HO-1-overexpressed Ad-MSCs was lower than that of Ad-MSCs. The mRNA expression levels of *TGF-β*, *HGF*, and *VEGF* did not differ between fresh and frozen-thawed Ad-MSCs, but *COX-2* and *IL-6* had significantly higher mRNA expression in frozen cells than fresh cells ($P < 0.05$). Fresh Ad-MSCs exhibited higher *HO-1* mRNA expression than frozen-thawed Ad-MSCs ($P < 0.05$), but HO-1-overexpressed Ad-MSCs did not differ from Ad-MSCs with respect to *HO-1* expression after thawing. The antioxidant capacity of HO-1-overexpressed Ad-MSCs was significantly higher than that of Ad-MSCs. Cryopreservation of Ad-MSCs negatively affects viability and antioxidant capacity, and HO-1-overexpressed Ad-MSCs might be useful to maximize the effect of Ad-MSCs for cytotherapy.

Keywords: Cryopreservation, Heme oxygenase-1, Mesenchymal stromal cells

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CONTENTS

I. Introduction

Mesenchymal stem cells (MSCs) are cytotherapeutic agents with great potential in the field of regenerative medicine to repair damaged tissue. Adipose-derived MSCs (Ad-MSCs) exhibit stable growth and proliferation during culture and potential differentiation to a variety of cells, including bone marrow stem cells (Dicker A et al.2005, Vieira NM et al.2010). Ad-MSCs are used to promote bone regeneration as well as in the treatment of neurological disorders such as spinal cord injury, stroke, and multiple sclerosis (Buschmann J et al. 2012, Jun YJ et al. 2006, Park KI 2000, Hintzen RQ 2002). Rather than direct conversion into differentiated cells, the repair mechanism is thought to involve the secretion of growth factors and promotion of the endogenous regenerative process by decreasing cell death and promoting nerve regeneration and revascularization (Chen J et al. 2003, Chen X et al. 2002, Chen J et al. 2003).

Although allogenic Ad-MSCs have an immunomodulatory effect, which is required for cytotherapy (Hare JM et al. 2009, Le Blanc K et al. 2008, Amado LC et al. 2005), they do not provide total immune evasion and thus the co-administration of immunosuppressive drugs needs to be considered (Ankrum JA et al. 2014). However, autologous Ad-MSCs may be able to completely evade a wide range of innate and adaptive immune systems (Eliopoulos N et al. 2005). However, the time required to collect, expand, and administer usable cells makes the application of Ad-MSCs difficult in patients with acute injuries. The key to successful clinical application of Ad-MSCs is to provide a sufficient quantity of Ad-MSCs in a timely manner. Frozen-thawed allogenic Ad-MSCs could serve as an alternative to overcome this limitation. Accordingly, it is necessary to evaluate the extent to which cryopreserved cells have the functional ability.

Heme oxygenase-1 (HO-1) is able to control the cell cycle and has cytoprotective, pro-

angiogenic, and anti-inflammatory properties (Ryter SW et al. 2006, Eisenstein RS et al. 1991, Suzuki M et al. 2003, Song R et al. 2003). The catabolism of heme provides cytoprotection via the induction of ferritin, antioxidative action of biliverdin and bilirubin, and anti-inflammatory effects of carbon monoxide.

This study was conducted to compare the viability, proliferation, antioxidant capacity, and mRNA gene expression levels of growth factors between canine Ad-MSCs and HO-1-overexpressed Ad-MSCs.

II. Methods

1. Isolation and cultivation of canine Ad-MSCs

Canine Ad-MSCs were obtained according to the method described in our previous paper (Ryu HH et al. 2009). Briefly, adipose tissues were aseptically collected from gluteal subcutaneous fat of 2-year-old beagle dogs (4 females). All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-141210-1), Korea. Adipose tissues (approximately 1 g) were washed extensively with phosphate-buffered saline (PBS), finely cut, and digested with collagenase type I (1 mg/m*l*; Sigma-Aldrich, St. Louis, MO, USA) for 2 h at 37 ℃. The tissue samples were washed with PBS solution and then centrifuged at 300 × *g* for 10 min. The stromal vascular fraction pellets were resuspended, filtered through a 100-μm nylon mesh, and incubated overnight in 10% fetal bovine serum (FBS, Gibco BRL, Grand Island, NY, USA) and Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Billings, MT, USA) at 37℃ with 5% humidified CO_2. After 24 hr, unattached cells and residual non-adherent red blood cells were removed by washing with PBS solution. The medium was changed at 2-day intervals until the cells became confluent. After the cells reached 90% confluence, they were subcultured. At passage 3, half of the cells were immediately used for in vitro experiments and the remaining half were stored in a -150℃ ultra-low temperature freezer and used after 2 weeks for the evaluation of the frozen-thawed cells.

2. Plasmid vector construction for canine HO-1

pPACK Packaging Plasmid Mix (System Biosciences, Mountain View, CA, USA) was used for lentiviral packaging. In brief, the gene encoding Flag-tagged HO-1 was amplified from cDNA of canine peripheral blood using Phusion DNA Polymerases

(Thermo Scientific, Pittsburgh, PA, USA) and a canine HO-1-specific primer set (Table 1) was inserted into a pCDH-EF1-MCS-pA-PGK-copGFP-T2A-Puro vector, with *Eco*RI and *Bam*HI restriction enzymes (System Biosciences).

3. Generation of lentivirus containing canine HO-1 and transfection into Ad-MSCs

HEK293T cells (Thermo Scientific) were maintained in 10% fetal bovine serum and 1% penicillin/streptomycin in DMEM at 37℃ and 5% CO_2. Twenty-four hours before transfection, 4×10^6 HEK293 cells were seeded into a 100-mm dish. The following day, 20 μl of lentiviral packaging mix (System Biosciences) encoding viral proteins Gag-Pol, Rev, and VSV-G and 2 μg lentiviral transgene plasmids were transfected into cells for lentivirus production using Turbofect (Thermo Scientific). Fourteen hours after transfection, the DNA reagent mixture was removed and replaced with 5% FBS in 14 m*l* of fresh DMEM. At 48 hr post-transfection, lentiviral supernatants were harvested and filtered with 0.45-μm filters. One volume of cold (4℃) PEG-it Virus Precipitation Solution (System Biosciences) was added to every 4 volumes of lentiviral particle-containing supernatant. The supernatant/PEG-it mixture was centrifuged at 1,500 × *g* for 30 min at 4 ℃. After the viral pellet was resuspended in 10 μl of cold (4 ℃) DMEM media, 100 μl of diluted viral particles (1×10^8 TU/m*l*) was added to the Ad-MSCs for the transfection and incubated in a T75 flask for 72 hr at 37 ℃; 10 m*l* of DMEM (10% FBS, 1% penicillin-streptomycin) was added before culture for 48 hr, and the colonies of cells were selected with puromycin (3 μg/m*l*). After culturing to passage 3, half of the cells were immediately used for the cell evaluation experiments, and the other half were cryopreserved and thawed after 2 weeks for evaluation.

4. Cryopreservation and thawing

Cells in a T175 flask were separated using 0.05% trypsin-EDTA (Gibco) and neutralized with DMEM after incubation for 15 min at 37 ℃, 5% CO_2. After centrifuging for 5 min at 900 × *g*, the supernatant was removed and 5×10^6 cells in cryogenic medium [50% DMEM, 40% FBS containing 10% dimethyl sulfoxide (DMSO)] were re-suspended. Since then, the cell suspensions were incubated at 4℃ for 1 hr, at -20℃ for 2 hr and at -80℃ overnight before moving the cryogenic vials to a -150℃ ultra-low temperature freezer (Davies OG et al. 2014). The cryopreserved cells were thawed after 2 weeks at 37℃ for 5 min for the recovery.

5. MTS assay

The cell viability and proliferation rate for fresh canine Ad-MSCs (MSCs), frozen-thawed canine Ad-MSCs (F-MSCs), fresh HO-1-overexpressed Ad-MSCs (HMSCs), and frozen-thawed HO-1-overexpressed Ad-MSCs (F-HMSCs) were compared by measuring 3-(4,5dimethylthiazol-2yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium using a commercially available kit (CellTiter 96® Aqueous, Promega, Madison, WI, USA). When the cells reached 80% confluence, attached cells were harvested with trypsin-EDTA and then immersed in a 96-well plate at a density of 1×10^4 cells/100 μl per well, and incubated for 0, 24, 48 and 72 hr. CellTiter 96 Aqueous One Solution Reagent (20 μl) was dispensed and the plate was cultured for 2 hr at 37℃ in a humidified 5% CO_2 atmospheric environment. Plates were read on a 680 micro-plate reader (Bio-Rad, Hercules, CA, USA) at a wavelength of 492 nm.

6. Observation of cell morphology

Cell morphology was observed 3 days after seeding using an inverted microscope (Olympus-ckx41, Tokyo, Japan), At passage three, 1×10^5 MSCs, F-MSCs, HMSCs, and F-HMSCs were seeded and grown in 6-well plates with DMEM medium containing 10% FBS.

7. Reverse transcription polymerase chain reaction

The total RNA was extracted using a Hybrid-R RNA Extraction Kit (GeneAll, Seoul, Republic of Korea) and the RNA concentrations were determined by measuring the absorbance at 260 nm using ImplenNanoPhotometer (model 1443, Implen GmbH, Munich, Germany). The Takara PrimeScript II First-strand cDNA Synthesis Kit (TaKaRa Korea, Seoul, Republic of Korea) was used to synthesize cDNA based on 1000 ng total RNA. One microliter of cDNA was amplified for polymerase chain reaction (PCR) analysis. PCR was performed with a final volume of 20 μl, which contained 10 μl of PCR Premix (EmeraldAmp™ PCR Master Mix, Takara, Otsu, Japan), 20 μM forward primer and 20 μM reverse primer (Standard Oligo, Bioneer, Daejeon, Korea), 3 μl of DNA (0.1–1.0 μg, diluted in TE buffer), and 3 μl of distilled water. The primers are shown in Table 1. Gene expression levels were compared after the target genes were normalized to the endogenous reference (GAPDH). cDNA templates were amplified for 27 to 35 cycles, and include denaturation at 94℃ (30 sec), annealing at 50–59℃ (30 sec), 72℃ (1 min), and a final extension at 72℃. The PCR reactions were performed using the T3000 Thermocycler (Whatman, Biometra, Biomedizinische Analtyik GmbH, Goettingen, Germany). After the reactions, 5 μl of PCR product was visualized using a 2% agarose gel (UltraPure™ Agarose, Invitrogen, Carlsbad, California, USA) by electrophoresis. Redsafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology Inc., Korea) was used

to stain a 2% agarose gel. The longitudinal sections of the visualized gel were analyzed using ImageJ (version 1.37, National Institutes of Health, Bethesda, MD, USA).

8. Total antioxidant capacity assay

The Total Antioxidant Capacity **(**TAC) Assay Kit (Cell Biolabs OxiSelect™, San Diego, CA, USA) was used to measure the antioxidant capacity of the cell extract. Cells were scrapped after washing them 3 times with PBS. The cells were suspended in cold PBS (1 × 10^7/m*l*), sonicated, and centrifuged for 10 min at 10,000 × *g* and 4 ℃. Twenty microliters of cell extracts were dispensed to the 96-well microtiter plate, and 180 μl of the 1× reaction buffer were added to each well and mixed. Fifty microliters of the 1× copper ion reagent were dispensed to each well to start the reaction, and incubated in a shaker for 5 min. Finally, 50 μl of 1× stop solution were added to end the reaction. The absorbance values were proportional to the total reductive capacity of the sample. Results are expressed as uric acid equivalents (UAE). A standard curve was used to determine the UAE (mM) of the sample, where $y = 0.462x + 0.061$ ($R^2 = 0.9938$); the *y*-value indicated the absorbance, which was used to obtain the UAE (mM) that provides the same OD at 490 nm.

9. Statistical Analysis

The results were expressed as means ± SD. The data were analyzed using the SPSS statistical program (version 20.0. IBM, Armonk, NY, USA). The Kruskal-Wallis test was used to assess differences among the groups. Mann–Whitney tests were carried out for the post-hoc test. A *P*-value of less than 0.05 indicated a significant difference between the groups.

III. Results

1. Viabilities of frozen-thawed Ad-MSCs

The absorbance of live MSCs was 0.43 ± 0.06 and that of F-MSCs was 0.33 ± 0.06 (n = 29), indicating that the viability of F-MSCs was less than that of MSCs (77%, $P < 0.01$). The absorbance of live HMSCs was 0.48 ± 0.11 and that of F-HMSCs was 0.34 ± 0.05 (n = 19), indicating that the viability of F-HMSCs was less that of HMSCs (71%, $P < 0.01$). Furthermore, there were no significant differences between MSCs and HMSCs or between F-MSCs and F-HMSCs. (Fig. 1).

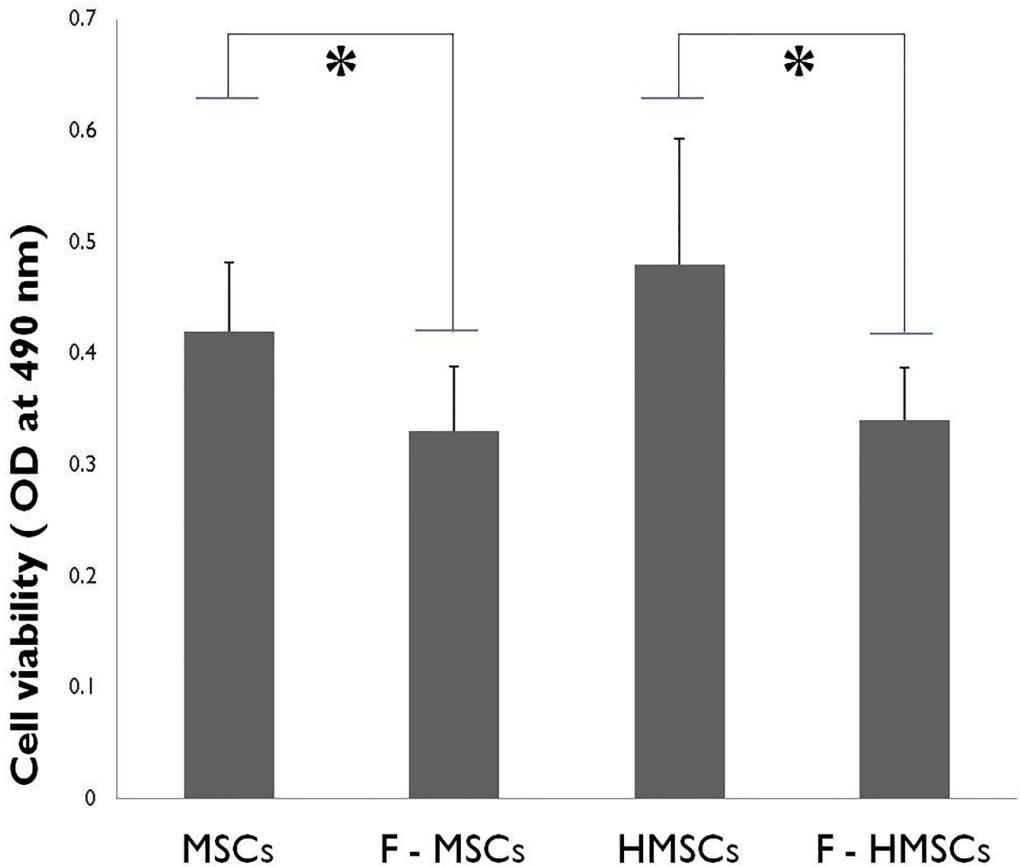

Fig. 1. Comparison of viabilities among fresh and frozen-thawed Ad-MSCs and HO-1-overexpressed Ad-MSCs. The viabilities of frozen MSCs and HMSCs were less than those of fresh MSCs and HMSCs, respectively.

All values are expressed as the means ± standard deviations and * indicates a significant difference between groups ($P < 0.05$).

MSCs, fresh canine Ad-MSCs; F-MSCs, frozen-thawed canine Ad-MSCs; HMSCs, fresh HO-1-overexpressed Ad-MSCs; F-HMSCs, frozen-thawed HO-1-overexpressed Ad-MSCs

2. Proliferation rates after culture of frozen-thawed Ad-MSCs

The proliferation rate (Fig. 2) of F-MSCs was lower than that of MSCs until 48 hr, after which the rate of F-MSCs increased abruptly and did not differ from that of MSCs at 3 days after culture. The proliferation rates of HMSCs after 3 days of culture were significantly lower than those of MSCs ($P < 0.05$).

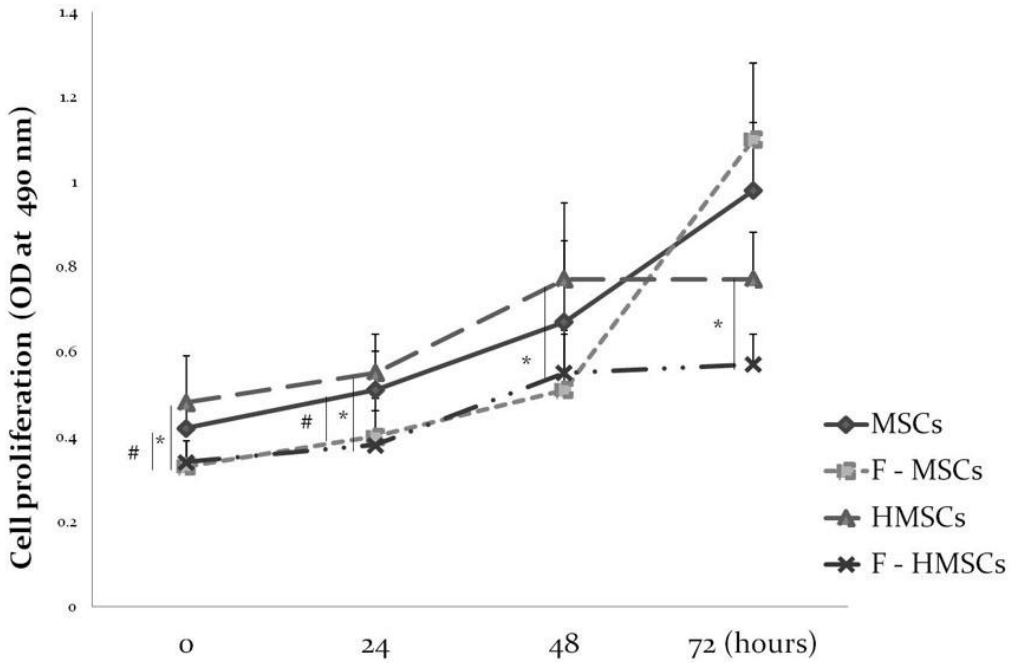

Fig. 2. Changes in proliferation rates of fresh and frozen-thawed Ad-MSCs and HO-1-overexpressed AD-MSCs. Proliferation rate of F-MSCs did not differ from that of MSCs at 3 days after culture but that of HMSCs were significantly lower than MSCs.
All values are expressed the means ± standard deviations and #,* indicates a significant difference between groups ($P < 0.05$). (#; between MSCs and F-MSCs, *; between HMSCs and F-HMSCs). MSCs, fresh canine Ad-MSCs; F-MSCs, frozen-thawed canine Ad-MSCs, HMSCs, fresh HO-1-overexpressed Ad-MSCs; F-HMSCs, frozen-thawed HO-1-overexpressed Ad-MSCs

3. Morphology of cells

The images taken 3 days after seeding (Fig. 3) showed approximately 60–70% confluency in MSCs and F-MSCs, with slim and spindle-shaped cells. Approximately 30–40% confluency was observed in HMSCs and 20–30% in F-HMSCs, showing delayed proliferation with more broad and spindle-shaped cells than were observed for MSCs.

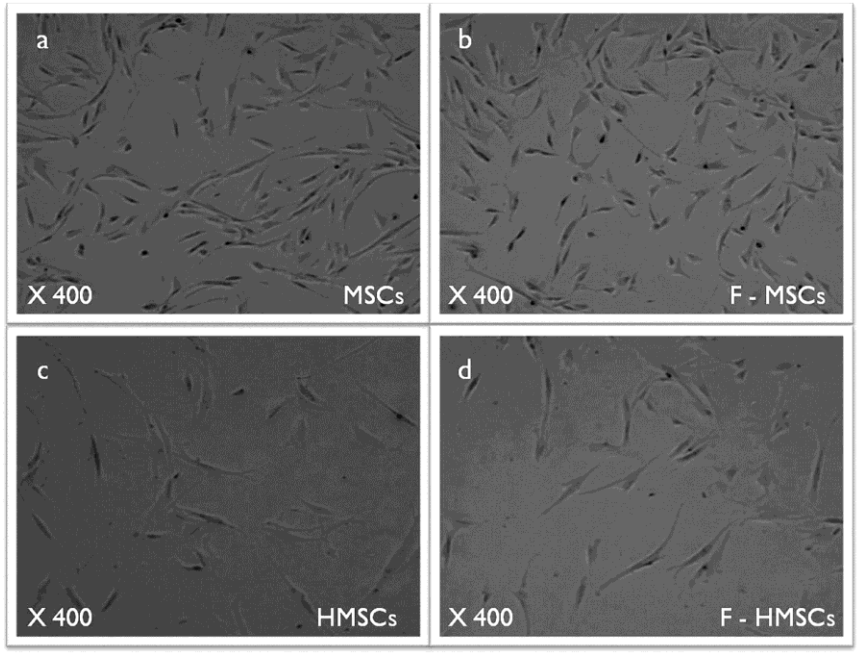

Fig. 3. Morphology of Ad-MSCs 3 days after seeding at passage 3. (400× magnification) (A) MSCs, 60–70% confluency, slim, spindle-shaped (B) F-MSCs, 60–70% confluency, slim, spindle-shaped (C) HMSCs, 30–40% confluency, broad, spindle-shaped (D) F-HMSCs, 20–30% confluency, broad, spindle-shaped.

4. mRNA expression levels of *TGF-β*, *HGF*, *VEGF*, *COX-2*, *IL- 6*, and *HO-1*

The mRNA expression levels of *TGF-β*, *HGF*, and *VEGF* did not show significant differences among MSCs, F-MSCs, HMSCs, and F-HMSCs. However, significantly higher *COX-2* and *IL-6* mRNA expression levels were observed in frozen-thawed cells including F-MSC and F-HMSCs than in fresh cells ($P < 0.05$). *HO-1* mRNA expression in MSCs was significantly higher than in F-MSCs ($P < 0.05$). *HO-1* mRNA expression in HMSCs was significantly higher than in MSCs ($P < 0.05$) but not higher than F-HMSCs (Fig. 4).

Table 1. Primers used in the PCR to detect mRNA of the canine Ad-MSCs

Target gene	Primer	Sequence	Size
Interleukin-6 (IL-6)	Forward	TCTGTGCACATGAGTACCAAGATCC	124 bp
	Reverse	TCCTGCGACTGCAAGATAGCC	
Cyclooxygenase-2	Forward	ACA TCC TGA CCC ACT TCA AG	387 bp
(COX-2)	Reverse	CAG GTC CTC GCT TAT GAT CT	
Transforming	Forward	CTC AGT GCC CAC TGT TCC TG	215 bp
growth factor beta	Reverse	TCC GTG GAG CTG AAG CAG TA	
(TGF- β)			
Hepatocyte growth	Forward	CCCGACAAGGGCTTTGATGA	873 bp
factor	Reverse	TCTGTTTCGAGAGGGGAAACAT	
(HGF)			
Vascular	Forward	CTACCTCCACCATGCCAA	785 bp
endothelial growth	Reverse	CATTGCCCTCAATGACCACT	
factor (VEGF)			
Heme oxygenase-1	Forward	GACAGCATGCCCCAGGAT	879 bp
(HO-1)	Reverse	TCACAGCCTAAGGAGCCAGT	
Glyceraldehyde 3-	Forward	CATTGCCCTCAATGACCACT	104 bp
phosphate	Reverse	TCCTTGGAGGCCATGTAGAC-3´	
dehydrogenase			
(GAPDH)			

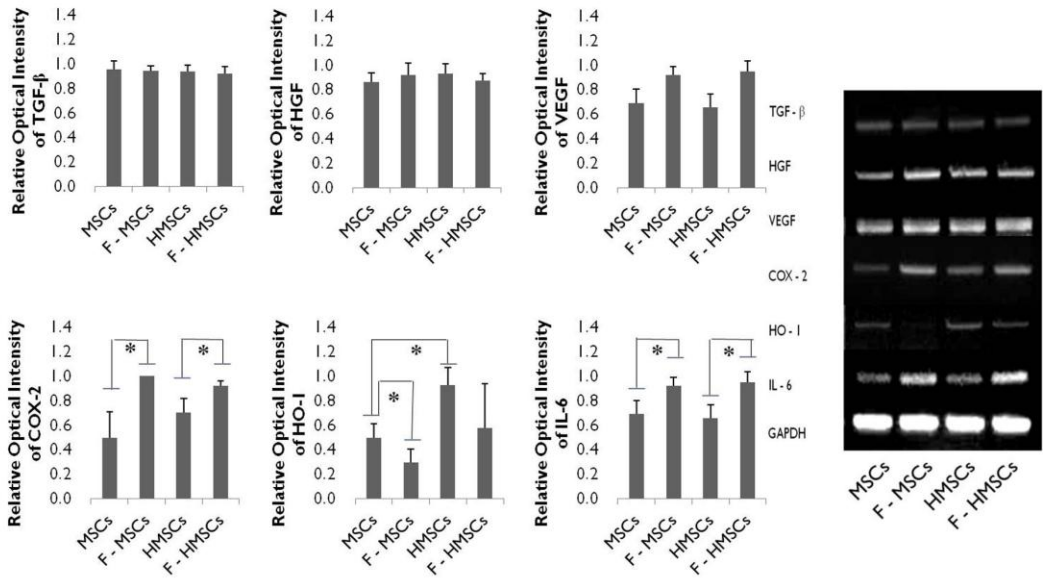

Fig. 4. Comparisons of mRNA expression levels of *TGF-β*, *HGF*, *VEGF*, *COX-2*, *IL-6*, and *HO-1* among MSCs, F-MSCs, HMSCs, and F-MSCs. The mRNA expressions levels of *TGF-β*, *HGF*, and *VEGF* did not show significant differences among groups but significantly higher *COX-2* and *IL-6* mRNA expression levels were observed in frozen-thawed cells ($P < 0.05$). *HO-1* mRNA expression in MSCs was significantly higher than that in F-MSCs but not than HMSCs and F-HMSCs. Each measure, expressed as means ± standard deviations, is expressed relative to the maximum value among groups. * $P < 0.05$

5. Analysis of total antioxidant capacity

HMSCs had higher antioxidant capacity than MSCs ($P < 0.05$). The total antioxidant capacities of F-HMSCs and F-MSCs were significantly lower than those of HMSCs and MSCs, respectively (85% for HMSCs; 66% for MSCs, $P < 0.05$). The antioxidant capacity of F-MSCs was significantly lower than that of F-HMSCs (44% for F-HMSC, $P < 0.05$) (Fig. 5).

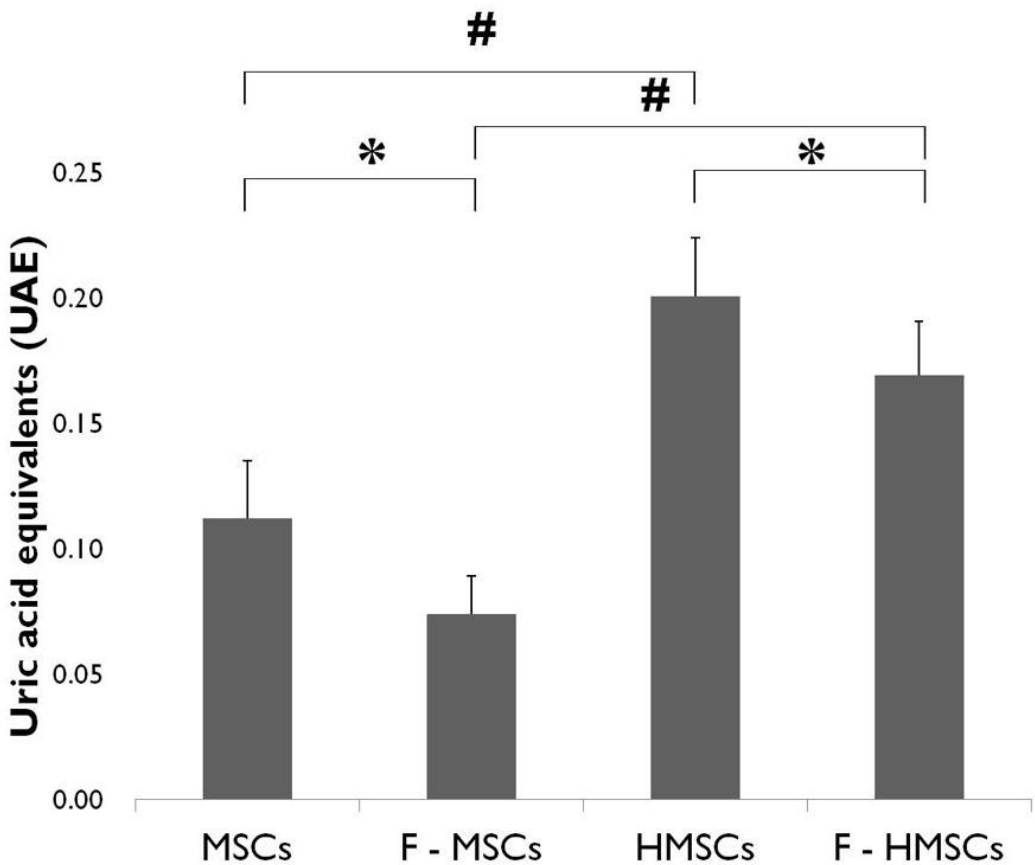

Fig. 5. Total antioxidant capacity (TAC) for MSCs, F-MSCs, HMSCs, and F-HMSCs. *, between MSCs and FMSCs, between HMSCs and FHMSCs; #, between MSCs and HMSCs, between F-MSCs and F-HMSCs ($p<0.05$)

IV. Discussion

Viability differed between fresh and frozen-thawed cells immediately after thawing. Some studies have shown that freeze-thawing can reduce cell viability (Pal R et al. 2008, Sohn HS et al. 2013). However, other studies have concluded that cryopreservation does not affect viability, morphology, or differentiation potency (Kotobuki N et al. 2005, Xiang Y et al. 2007). It has been suggested that the conflicting results reflect the different methods used to measure cell viability. Our study evaluated viability by MTS assay, which measured biological activity, rather than by a simple assessment of cell status (i.e., live or dead). Our findings confirmed that the viability of frozen-thawed cells was about 70% relative to that of fresh cells.

The proliferation rates of fresh and frozen-thawed HO-1-overexpressed Ad-MSCs were lower than those of intact Ad-MSCs after 3 days of culture. HO-1 activity is associated with enhanced cell survival, proliferation, and migration, and a reduced inflammatory response (Schipper HM et al. 2009, Kozakowska M et al. 2012, Laumonier T et al. 2008). However, it was reported that overexpressed HO-1 activity in vascular smooth muscle cells results in a slower growth rate than that of wild-type vascular smooth muscle cells (Zhang M et al.2002). Ninety one percent of human HO-1-transfected cells were in the growth-arrested phase of the cell cycle (G0/G1). Although reduced proliferation of overexpressed-HO-1 Ad-MSCs in the present study was observed, the total antioxidant activity was significantly higher than that of fresh Ad-MSCs as well as frozen Ad-MSCs. If the benefits of Ad-MSC therapy involve indirect environmental modification via anti-oxidation, anti-inflammation, and anti-apoptosis effects rather than direct differentiation (Ruff CA et al. 2012), frozen overexpressed-HO-1 Ad-MSCs may be useful.

VEGF secreted by Ad-MSCs is involved in the control of nerve regeneration as well as the maintenance and survival of newly created blood vessels (Jin K et al. 2002,

Schanzer A et al.2004). In addition, VEGF, HGF, and other various growth factors behave as neuroprotectors (Kim HJ et al. 2010, Sasaki M et al. 2009). In this way, growth factors such as HGF, TGF-β, and VEGF are important for healing damaged tissue. In the present study, there was no detectable difference in the mRNA expression of growth factor genes between MSCs, F-MSCs, HMSCs, and F-HMSCs. However, the inflammatory factors *COX-2* and *IL-6* in frozen Ad-MSCs has increased expression. Heat stress during the freeze-thaw process promotes *COX-2* expression (Rossi A et al. 2012), and can enhance the expression of *IL-6* (Vekataraman M et al. 1994, Hamzic N et al. 2013). Therefore, the inflammatory response is likely to occur when frozen-thawed cells are used.

HO-1 increases the survival of Ad-MSCs in acute myocardial infarction (Tang YL et al.2005), and may control the differentiation of chondrocytes, neurons, and osteoblast (Kozakowska M et al.2014, Vanella L et al.2010). In addition, HO-1 has an effect on blood flow recovery and nerve function recovery (Suzuki M et al. 2003). Our study showed that HO-1-overexpressed Ad-MSCs have higher antioxidant capacity than Ad-MSCs, regardless of cryopreservation.

In clinical trials, the use of cryopreserved products immediately after thawing fails more often than the use of fresh Ad-MSCs (François M et al. 2012). In the present study, frozen-thawed Ad-MSCs were limited as a therapeutic tool owing to reduced viability, lower *HO-1* mRNA expression, and lower total antioxidant activity relative to fresh cells. These results suggested that frozen-thawed HO-1-overexpressed Ad-MSCs are an alternative source for cytotherapy.

V. References

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VI. 국문초록

동결해동 후 개의 헴옥시게네이즈-1 유전자 과발현 세포와 일반 지방유래 줄기세포 생존성 및 항산화능 비교연구

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동종 지방유래 중간엽 줄기세포는 항염효과와 항산화효과를 통해 자가 중간엽 줄기세포를 이용한 세포치료에 대안이 될수 있다. 동종의 동결 해동 지방유래 중간엽 줄기세포는 이런 목적을 위해 즉각적으로 사용될 수 있는데 동결 해동 세포에 대한 생존성이나 기능에 대해서는 아직 명확히 평가되지 않았다. 본 연구는 일반 중간엽 줄기세포와 헴옥시게네이즈-1 유전자를 과발현 시킨 중간엽 줄기세포에 대하여 신선세포와 동결해동 세포로 나누어

그 생존성 및 기능을 비교해보고자 하였다. 따라서 생존성, 증식능, 항산화능과 성장인자에 대한 mRNA발현능을 나누어 평가하였으며 그 결과 일반 지방유래 줄기세포와 헴옥시게네이즈-1 유전자를 과발현 지방유래 줄기세포 둘다 신선세포에 비해 동결해동 세포에서 생존성이 떨어짐을 확인할수 있었다(동결해동 지방유래줄기세포 77%, 동결해동 헴옥시게네이즈-1 유전자 과발현 지방유래 줄기세포 71%). 그러나 배양 3일 후 증식능의 경우 일반 지방유래줄기세포는 신선세포와 동결해동 세포가 세포 배양 3일 후 차이를 보이지 않았다. 반면 헴옥시게네이즈-1 유전자 과발현 지방유래 줄기세포의 경우는 신선세포와 동결해동세포가 모두 일반 지방유래 줄기세포보다 증식률이 떨어짐이 확인되었다. 유전자 발현능에 대해서는 *TGF-β*, *HGF*, *VEGF*는 유의적인 차이를 보이지 않았으나 *COX-2*와 *IL-6*의 경우는 신선세포보다 동결해동 세포에서 더 높게 나타났다. 일반신선세포는 일반동결해동 세포보다 더 높은 *HO-1* 발현을 보였으나 헴옥시게네이즈-1 유전자 과발현 신선세포와 동결해동세포는 차이를 보이지 않았다. 또한 헴옥시게네이즈-1 유전자 과발현세포는 일반 지방유래줄기세포 보다 항산화능이 높게 나타났다.

일반 지방유래줄기세포의 동결보존은 줄기세포의 생존성과 항산화능에 대해 부정적인 영향을 미칠수 있으며 헴옥시게네이즈-1 유전자 과발현 지방유래줄기 세포는 지방줄기세포를 이용한 세포 치료에 있어서 유용한 재료가 될 가능성이 있다.

주요어 : 동결보존, 헴옥시게네이즈-1, 중간엽기질세포

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