Research Article
Volume 8:3, 2020

Dermatology and Dermatologic Diseases

ISSN: 2329-6895

Open Access

Safety and Efficacy of Subcutaneous Infusion of Human Adipose Tissue-Derived Mesenchymal Stem Cells in the Treatment of Mouse Atopic Dermatitis: A Pilot Study

Yeganeh Davoudi[1], Minoo Mahmoodi[1]*, Masoud Ghorbani[2], Mohammad Ali Nilforoushzade[3,4], and Rahim Ahmadi[1]

[1]*Department of Biology, Faculty of Sciences, Islamic Azad University, Hamadan Branch, Hamadan, Iran*
[2]*Pasteur Institute of Iran, Departtment of Research and Development, Research & Production Complex, 25 Kilometers of Karaj Highway, Karaj, Iran*
[3]*Tehran University of Medical Sciences, Skin and Stem Cell Research Center, Tehran, Iran*
[4]*Jordan Dermatology and Hair Transplantation Center, Tehran, Iran*

Abstract

Background: Atopic dermatitis (AD) is a type of inflammation of the skin. It results in itchy, swollen, red, and cracked skin. Mesenchymal Stem Cells (MSCs) could suppress allergic responses in AD. Indeed, the immunomodulatory ability of MSCs can be usefully applied for the treatment of autoimmune and inflammation – related diseases such as asthma, rhinitis and dermatitis. We aims to evaluate safety and efficacy in DNFB – induced atopic dermatitis mouse model after a subcutaneous infusion of xenogeneic human Adipose Tissue – derived MSCs (hAT-MSCs). Therefore, hAT-MSCs have possibility as a new drug for AD.

Keywords: Mesenchymal stem cells • Atopic dermatitis • Stem cell therapy • Subcutaneous • Safety

Introduction

Mesenchymal Stem Cells (MSCs) have an inherent ability for self-renewal, proliferation, and differentiation toward mature tissues, depending on the surrounding microenvironment. Such characteristics intrinsic to stem cells make MSCs very attractive for use in the cell therapy and regenerative medicine. MSCs are found in various tissues, including Bone Marrow (BM) [1], umbilical cord blood [2], placenta [3], and fat [4]; these tissues contain a rare population of adult stem cells that have the potential to undergo multilineage differentiation into osteoblasts, adipocytes, and chondroblasts *in vitro* [5].

The MSCs comprise only a minor fraction of BM and other tissues, with Bone Marrow MSCs (BMMSCs) constituting a mere 0.0001%-0.01% of all BM-nucleated cells [6]. In contrast, adipose tissues contain 1×10^5 MSCs in each gram of fat [7]. Furthermore, the differential capacity of Adipose Tissue-derived MSCs (AT-MSCs) is less affected by the donor age [8]. Adipose tissue is an accessible, abundant, and reliable site for the isolation of adult stem cells suitable for tissue engineering and regenerative medicine applications. In this regard, the treatment efficacy of AdMSCs for various diseases has been reported using animal models. Another interesting characteristic of MSCs is their ability to mobilize to areas of tissue injury. Localized abdomen irradiation significantly enhances MSC homing specifically to radiation-injured tissues of mice [9]. The potential for the minimally invasive delivery of MSCs via systemic infusion is of particular interest because of the easy, minimally invasive application of stem cells. Before culture-expanded stem cells can be used in the human clinic, Good Manufacturing Practices (GMP) must be developed for the production of clinical-grade human stem cells. The stem cells should be evaluated for aerobic and anaerobic bacteria, endotoxin, and ESR. In This study safety of the stem cells, including endotoxin and ESR, was confirmed. Finally, the animal's models were administered high dose (0.75×10^5 cells per patient) of xenogeneic hAT-MSCs subcutaneously and monitored the safety as well as the efficacy.

***Address for Correspondence:** Minoo Mahmoodi, Department of Biology, Faculty of Sciences, Islamic Azad University, Hamadan Branch, Hamadan, Iran. Tel: +989183138358. E-mail: minoomahmoodi@yahoo.com*

Received 22 February, 2021; **Accepted** 23 March, 2021; **Published** 30 March, 2021

Materials and Methods

Isolation and culture of hADMSCs

Human adipose tissues were obtained by simple liposuction from the abdominal subcutaneous fats with an informed consent. Subcutaneous adipose tissues were digested using 1 mg/ml collagenase I (Gibco (UK)) under gentle agitation for 60 min at 37°C. The digested tissues were filtered through a 100 µm nylon sieve to remove cellular debris and were centrifuged at 470 g for 5 min to obtain a cell pellet. The cells were resuspended in Dulbecco´s modified Eagle´s medium (DMEM; Invitrogen) based media containing 0.2 mM ascorbic acid and 10% Fetal Bovine Serum (FBS) (Gibco (UK)). The cell suspension was then re-centrifuged at 470 g for 5 min. The supernatant was discarded and the cell pellet was collected. The cell fraction was cultured overnight at 37°C/5% CO_2 in DMEM-based media containing 0.2 mM ascorbic acid and 10% FBS. After 24h, the cell adhesion was checked under an inverted microscope, and non-adherent cells were removed by washing with phosphate-buffered saline (PBS). The cell medium was changed to Keratinocyte-SFM (Invitrogen) based media containing 0.2 mM ascorbic acid, 0,09 mM calcium, 5 ng/ml rEGF, and 5% FBS. The cells were maintained for 4–5 days until confluent (passage 0). When the cells reached 90% confluency, they were sub cultured and expanded in keratinocyte-SFM-based media containing 0.2 mM ascorbic acid, 0.09 mM calcium, 5 ng/ml rEGF, and 5% FBS until passage 3. FBS contaminant from cultured MSCs, were completely removed by several washing with PBS. Aliquots of the hAdMSCs were then tested for cell viability and fungal, bacterial, endotoxin, and *mycoplasma* contamination as demanded by the LAL kit, PCR, before further use. The procedure for hAdMSCs preparation was performed under the Skin and Stem Cell Research Center (SSRC) regulations at the Tehran University of Medical Sciences.

Flow cytometry analysis

The flow cytometric characterization of MSCs was performed using the following monoclonal antihuman antibodies including CD90, CD34, CD45 and CD44 conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE). All antibodies were purchased from the Abcam (USA) company. FACSort

(Becton Dickinson, Sanjose, CA) was used for sample acquisition and FACS Becton 6.1 software was recruited for the data analysis. Cells were trypsinized, after reaching 90% confluence, and re-suspended in DPBS (Invitrogen). Approximately 10^6 cells/ml were incubated in dark with labeled antibodies at 37°C for 1 h. Cells were then characterized using the following conjugated monoclonal antibody combinations: CD34/ CD45/ CD90/ CD44. Negative controls of FITC- or PE- conjugated corresponding immunoglobulins were used. Samples were then washed and acquired immediately after staining. A total 100,000 events were acquired, at low speed, after the cytometer was compensated and calibrated.

In vitro differentiation

For osteogenic induction, hAdMSCs cells were plated at 1×10^5 cells/ml in Keratinocyte-SFM-based media containing 0.2 mM ascorbic acid, 0.09 mM calcium, 5 ng/ml rEGF, and 5% FBS. The medium was replaced with osteoblast induction medium (NH Osteodiff medium) or control medium. The cells were maintained in culture for 14 days, with 90% of the media being replaced every 3 days. Osteogenic differentiation was detected by Alizarin red S staining (Stem Cell Technology Company).

Chondrogenic induction Was performed by plating 2.5×10^5 hAdMSCs cells/ml in Keratinocyte-SFM-based media containing 0.2 mM ascorbic acid, 0.09 mM calcium, 5 ng/ml rEGF, and 5% FBS. Cells were centrifuged at 500 g for 5 min, re-suspended in chondrogenic induction medium (NH chondrogenic medium), and centrifuged again at 500 g for 5 min to form pellets. The pellets were maintained in culture media using polypropylene tubes for 14 days, with 50% of the media being replaced every 3–4 days. Chondrogenic differentiation was assayed by toluidine blue O staining.

Detection of *mycoplasma*

Many methods are available for the detection of *mycoplasma*. We used PCR in this study (Table 1). Cells for testing should be prepared fresh whenever possible, as storage or snap freezing may reduce the number of viable organism. Appropriate positive and negative controls must always be included in PCR assay.

Polymerase chain reaction analysis

Polymerase chain reactions (PCR) of hAdMSCs at different passages (0, 3, 6 or 9) were analyzed.

The cell cultures were obtained from hAdMSCs bank at the Skin and Stem Cell Research Center (SSCRC). Totally, 3 samples of hAdMSCs have been checked for *mycoplasma* contamination.

DNA extraction and nucleotide sequences of primers

Boiling method was used to extract DNA. Briefly, 100 µl of the cell suspension in mineral Oil was placed in boiling jar for 10 minutes. The cells were placed on ice to cool down and centrifuged at 12000 g for 10 minutes; the supernatant was then transferred to a new tube and used as template in PCR testing. The target gene was 16srRNA of *mycoplasma* using the following primers [GPO3 5´- GGGAGCAAACACGATAGATACCCT- 3´ and MGSO 5´ - TGCACCATCTGTCACTCTGTTAACCTC - 3´] (Table 2). The PCR reaction consisted of 2 µl of template DNA, 25/0 µl of each one of forward and reverse primers, 2 µl of PCR buffer (10x), 5/2 µl $MgCl_2$, 5/0 µl of dNTP mixture (10 mM) and 2 µl of Taq Enzyme (Taq DNA polymerase 2X Master Mix Red (Ampliqon, Denmark)) and 5/15 µl of double distilled and deionized water. The thermo cycler was programmed for 40 cycles at 94°C for 20 s, 58°C for 20 s, and finally 70°C for 30 s (Tables 3 and 4). The PCR products were electrophoresed in agarose gel and visualized by UV transilluminator after staining the gel with ethidium bromide. Serial dilutions of *Mycoplasma* arginine suspension with specific CFU was assayed to check the sensitivity of PCR.

Passaging and cell proliferation analysis

To determine the proliferation rate, a total of 1,000 cells/cm^2 were seeded in T-25 flasks. Each time point and the passage had three replicates. Cells were trypsinized after reaching 90% confluency. The cell numbers were then counted in each passage and assessed for viability using trypan blue dye. Cells were re-plated for subsequent passages and a total of ten passages were performed in this study.

Cell proliferation assay using MTT

To correlate between the expression of cell proliferation, a colorimetric 3-(4,5- dimethylthiazol - 2y1) 2,5 diphenyltetrazolium bromide (MTT) (sigma-Aldrich) assay was used. The MTT assay, that replaced the radioactive tritiated thymidine incorporation assay, was the first widely accepted method to measure cell proliferation. After 4 days of culture, MTT solution was added and the cells were incubated in 5% CO_2 humidified incubator at 37°C for 4 h. Then the formazan solubilization solution was added and the absorbance was detected at 450 nm (Tables 5-8).

Quality control test

Contamination of the gram negative bacterial endotoxin was detected on

Table 1. *Mycoplasma* detection method, sensitivity and advantage and disadvantages.

Method	Sensitivity	Advantage	Disadvantage
PCR	High	Rapid	Requires optimization

Table 2. Primers used for fragment amplification of the 16srRNA gene of *Mycoplasma* by PCR.

Species	primers sequence	fragment size
Mycoplasma		
Primer – GPO3	5'- GGG AGC AAA CAC GAT AGA TAC CCT – 3'	270 bp
Primer – MGSO	5' – TGC ACC ATC TGT CAC TCT GTT AAC CTC – 3'	

Table 3. PCR amplification with specific primers for Mycoplasma.

PCR program		Mycoplasma
Initial Denaturation		94°C (4 min)
40 cycles	Denaturation	94°C (40 min)
	Annealing	58°C (60 min)
	Extensions	70°C (50 min)
Final Extensions		72°C (7 min)

Table 4. Immunophenotype of culture – expanded human mesenchymal stem cells at passage 3

Surface markers	The positive percentage (%) (n = 8)
CD34	99.8 ± 0.197
CD44	2.22 ± 97.8
CD45	99.8 ± 0.206
CD90	4.15 ± 95.8

Data are expressed as mean ± standard deviation.

Table 5. Viability (%) of hAdMSCs after 24 hours by MTT assay

Repetition	1	2	3	4	5	control
Viability (%)	96/66	88/80	95/55	93/ 14	92/14	96

Table 6. Viability (%) of hAdMSCs after 48 hours by MTT assay

Repetition	1	2	3	4	5	control
Viability (%)	90/5	89/87	89/69	89/56	91/05	96

Table 7. Proliferation rate of hAdMSCs after 24 hours by MTT assay

Repetition	1	2	3	4	5
			Control		
Proliferation rate	1/493	1/6	1/44	1/6	1/546
			96		

Table 8. Proliferation rate of hAdMSCs after 48 hours by MTT assay

Repetition	1	2	3	4	5	control
Proliferation rate	2/186	2/4	2/346	2/293	2/346	96

the stored hAdMSCs, using the LAL kinetic–kit (lonza). LAL test is a quantitative method to detect Gram negative bacterial endotoxin in a solution. LAL is an aqueous extract of blood cells (amebocytes) from the "horseshoe crab" limulus Polyphemus. The endotoxin catalyzes the activation of a proenzyme in the LAL. The rate of the reaction depends on the concentration of endotoxin presence. Standard curve with 0.005 endotoxin unit EU/ml was used in this assay. Determination of detectable endotoxin was performed by the high and low points of a valid standard cure. The correlation coefficient (CC) of the calculated standard curve should be ≥0.980. In our assessment evaluation on 100 µl supernatant by incubating the hAdMSCs and the calibrators at 37°C in the presence of the LAL for 1 hour and 40 minutes in a microplate reader ELX (lonza).

Determination of erythrocyte sedimentation rate (ESR)

The erythrocyte sedimentation rate (ESR) is a commonly used test to screen for inflammatory conditions such as infections, autoimmune diseases, and cancers. The modified Westergen method (requires collecting 2 ml of venous blood into a tube containing 0.05 ml of sodium citrate) was used for the measurement of erythrocyte sedimentation rate. Four volumes of the blood (anticoagulated blood with EDTA) sample and 1 volume of the normal saline were mixed (Wetergen, 1957). The blood was then placed glass Westergen pipette and allowed to stand for 1 h in a vertical position. The number of millimeters the red blood cells fall during this time represented the ESR.

Statistical analysis

The continuous variables are presented as mean ± SD. All statistical comparisons were made using one–way ANOVA followed. $P<0.05$ was considered statistically significant. Analyses were performed using SPSS software.

Results

Morphology of hAdMSCs during culture-expanded

hAdMSCs were microscopically observed at every passage. We observed cell deformation up to passage 4. Cells with spindle–shaped and a fibroblast–like morphology were checked 24-48 h after isolation and kept to passages 4 (P4). These characteristics were well preserved during repeated subculture. hAdMSCs Showed ability to form multilayer after being confluent. Characterization of hAdMSCs culture–expanded cells at passage 4 were prepared from 8 donors, and later on the portion of enlarged cells with changed morphology was gradually increased, the volume of the hAdMSCs increased gradually with increasing passage number, as shown in Figure 1.

Immunophenotypic characterization of culture – expanded hAdMSCs at passage 3

Immunophenotypic characterization of the hAdMSCs, culture–expanded cells at passage 3 was prepared from 8 donors, and surface protein expression was examined by flow cytometry. Flow cytometric analysis revealed that MSCs were identified based on several markers such as CD34, CD90, CD44 and CD45. The hAdMSCs were positive for CD90, CD44, but were negative for CD34, CD45, as shown in Figure 2. The results of FACS analysis on 8 different donor samples are listed in Table 4.

Characterization of cell differentiation

The ability of hAdMSCs to differentiate into various cell types was evaluated. Differentiation of hAdMSCs was confirmed using the human commercial differentiation media (Gibco, Grand Island) (Figure 3). Culture–expanded cells at passage 3 showed the ability of *in vitro* differentiation into osteoblasts as assessed by Alizarin red S staining (Figure 3A), and into chondroblasts as assessed by toluidine blue O staining (Figure 3B). Cells undergoing chondrogenic differentiation appeared blue when stained with toluidine blue and undergoing osteogenic differentiation appeared red when stained with Alizarin red.

Results of the PCR

The PCR results were interpreted by comparing the presence and size of the PCR product from test samples with those of the positive control reaction. The positive control and the inhibition control both showed a 270-bp band, whereas the negative controls showed no bands in this region (Figure 4). The test was only accepted as a valid test when the negative controls were negative and both the positive control and the inhibition control revealed appropriate bands. In this study, PCR products next to the size marker, positive and negative control using electrophoresis, on 1% *mycoplasma* agarose gel was used as a control. Using specific *mycoplasma* species primers, The PCR test was performed with applied changes and a dedicated single band was observed in the 270-bp region.

Cell viability

To evaluate the viability of the cultured hAdMSCs cultured different passages, the MTT assay was used. The representative absorbance of the hAdMSCs is shown in Tables 5 and 6. The cell viability at 48 hours of incubation decreased as compared with 24 hours of incubation. This result indicates that cell viability decreased with increasing the age of cells in culture.

Proliferation of the hAd-MSCs

Significant increase in proliferation was observed in passage 1 as compared with passage 2, indicating more rapid rate of proliferation at early stages. Based on these results, early passages, (P1) and (P2), tended to double their population in 24 hours and 48 hours as shown in Tables 7 and 8 (Figure 5).

Endotoxin test

The endotoxin test was performed once times, under operating conditions by the QC manager. Endotoxin concentration in 250 µl of samples, it was less than 0/43 EU/ml. Pyrogen–free water used as a negative control, had an endotoxin value less than the lowest standard according to the kit LAL protocol (lonza).

ESR test

The purpose of this section aimed to define treatment-associated adverse events and biological efficacy measure by ESR response criteria and

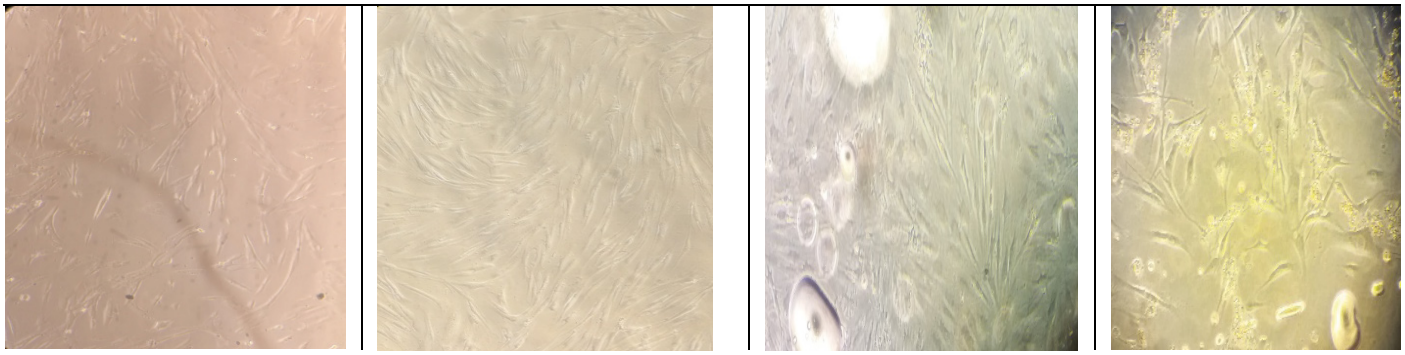

Figure 1. Morphology of the hAdMSCs during *in vitro*. Images of the hAdMSCs in 4 passages with 90% confluence were shown. The hAdMSCs show ability to form multilayer after confluent. The hAdMSCs gradually differentiated into polygonal cells with long protrusions.

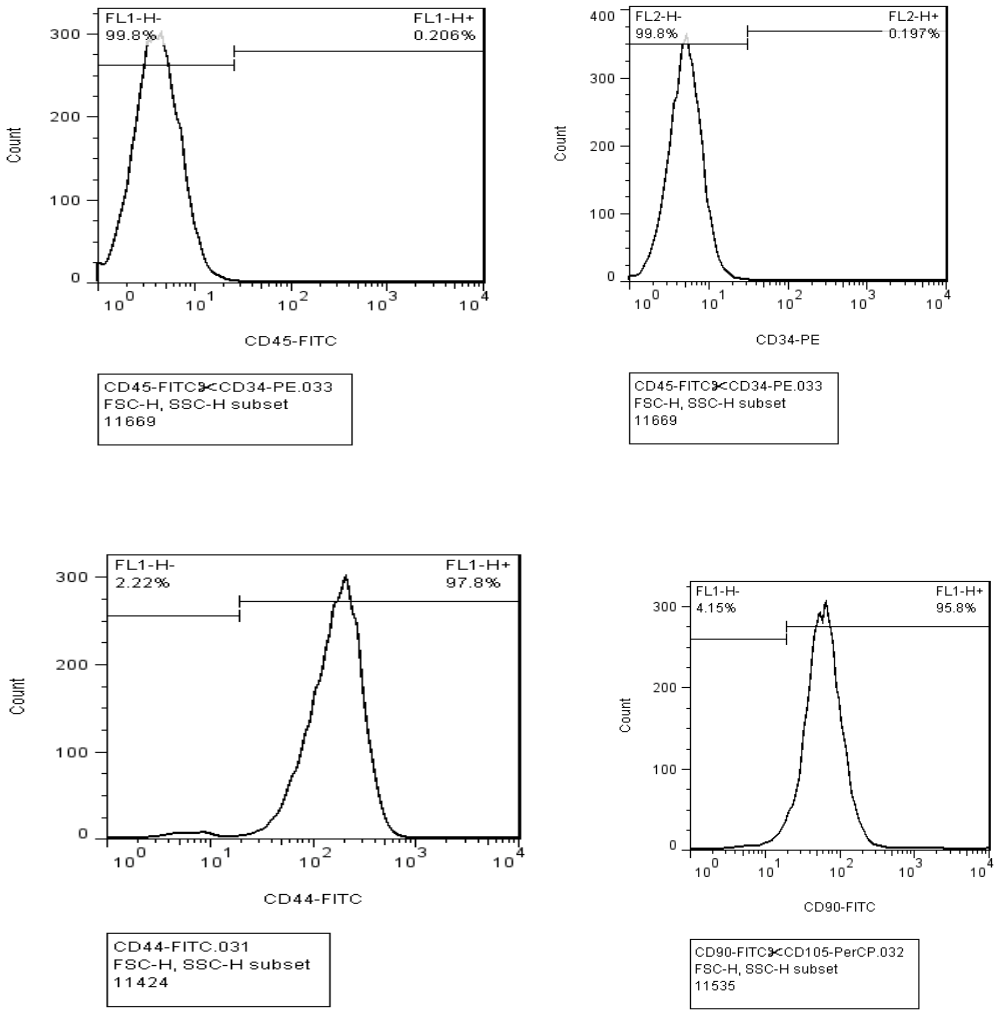

Figure 2. Immunophenotypic characterization of the *in vitro* cultured hAdMSCs. Representative linear plots of cytometric analysis against CD90, CD44, CD34 and CD45 for passage 3. Flow cytometric analysis was performed by gating on the CD34, CD45 negative population as shown above.

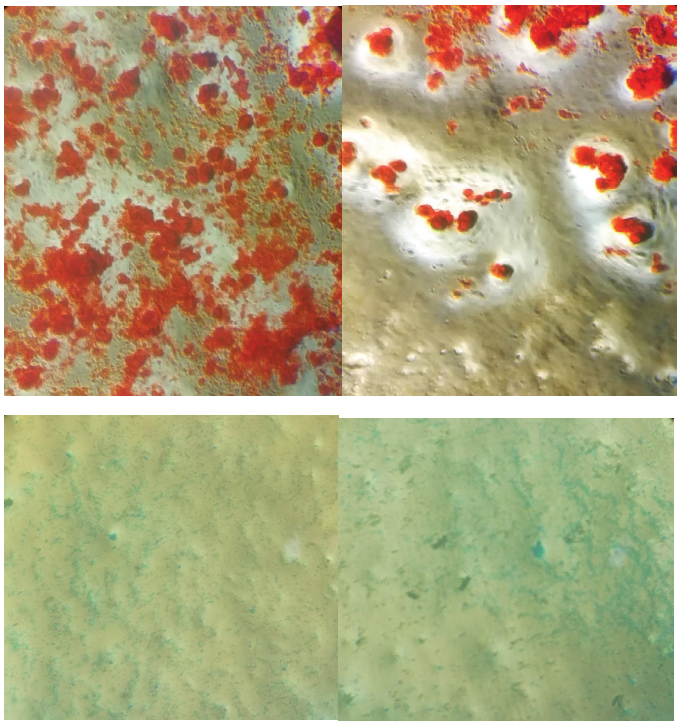

Figure 3. Multilineage differentiation of culture -expanded hAdMSCs (20x), **(A)** at day 14 after differentiation into osteoblasts. Calcium deposits stained in red in differentiated cells (Alizarin Red staining). **(B)** Image of hAdMSCs (10x), at day 21 after differentiation into chondrocytes.

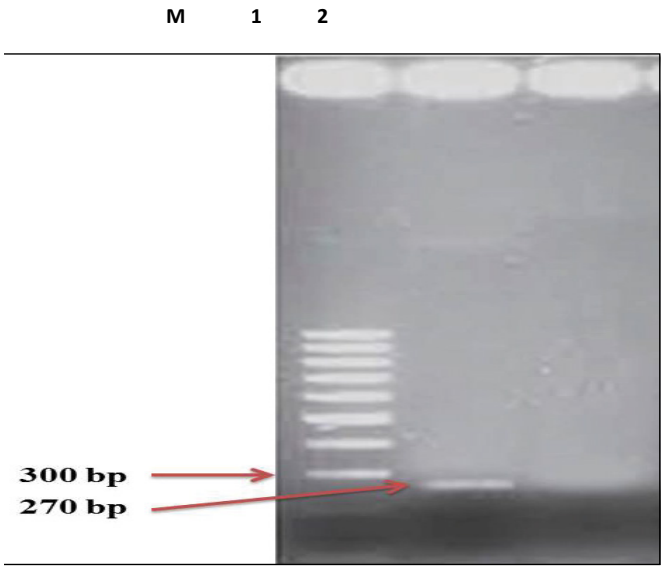

Figure 4. Typical gel photo from direct PCR. Evaluation of the mycoplasma PCR product in tested samples. Numbering from left to right, Lane M) marker 100- bp DNA ladder), Lane 1) positive control (standard sample band, MS-NCTC 10124-05 in the 270- pb region), Lane 2) negative control.

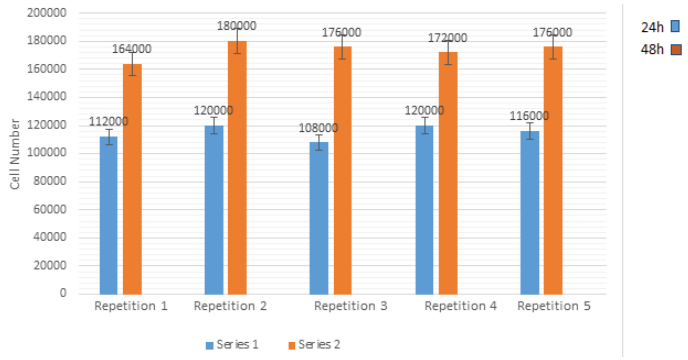

Figure 5. The number of hAdMSCs in two day, during incubation times 24 and 48 hours by MTT assay.

immunological parameters 2 weeks post infusion. In addition, to evaluate the safety and efficacy of hAdMSCs therapy up to 2 weeks post infusion (Figure 6).

Discussion

The use of MSCs cells from adipose tissues is a new concept technology that breaks the limits of current drug and medical technologies. Adipose tissue has been demonstrated to be a viable source of MSCs in numerous species, including dogs and humans [10-15]. In regenerative medicine, stem cells can replace injured cells and tissues. They can also be used to address various incurable diseases that cannot be adequately treated with medication or surgery, including critical limb ischemia, arthritis, and atopic dermatitis. This study was designed to evaluate the clinical effects of AD-MSC therapy on atopic dermatitis. To achieve therapeutic clinical results with MSCs, large quantities of the cells must be generated by *in vivo* expansion. However, long – term culture can alter the quality of MSCs, including their proliferative capacity [16], differentiation potential [17], and trophic activity [18]. With regard to the safety of culture-expanded stem cells, researchers must verify that the passaged MSCs are genetically stable. They must also obtain consistent data on the morphological, immunophenotypic, and differentiation characteristics, as well as data concerning their toxicity and tumorigenicity. We demonstrate for the first time that subcutaneous administration of xenogeneic hAd-MSCs, did not exhibit any embolism–related, clumped cells–related symptoms, supporting the safety of hAd-MSCs. MSCs migrate preferentially to sites of injury and inflammation, where they promote functional recovery of tissue as

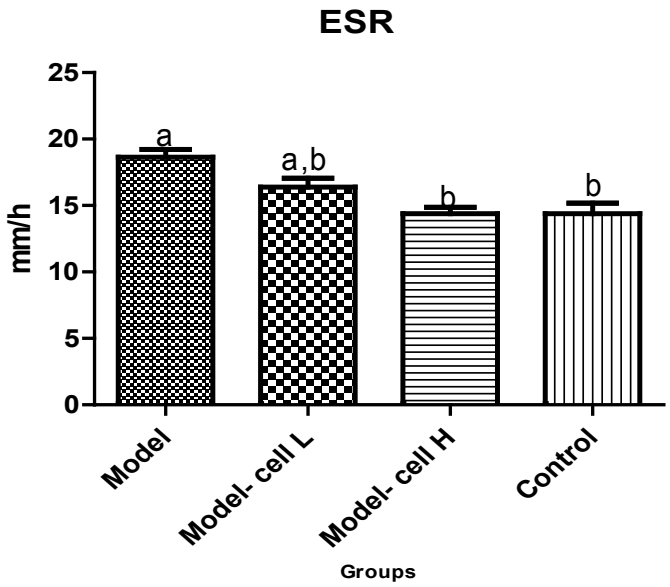

Figure 6. Two weeks after infusion, the erythrocyte sedimentation rate (ESR) of the laboratory groups was measured. As shown ESR was reduced 24h in the group infused with the higher dose of hAdMSCs (0/75 × 10^5 hAdMSCs per subject) compared to other groups.

well as a decrease in inflammation [19-22]. We evaluated the safety of AdMSCs obtained from human adipose tissues in animal models. The hAd-MSCs were attached to the bottom of a culture dish, where they grew rapidly and acquired a spindle shape. More than one billon cells were easily obtained at passage 3 or 4 from <10 gr of fat tissue. The immunophenotype of hAdMSCs showed the expression of MSC antigens (CD44, CD90) and the absence of hematopoietic and endothelial antigens (CD34, CD45). The hAdMSCs were capable of differentiating into multiple lineages, including adipogenic, osteogenic, chondrogenic, myogenic and neurogenic. Systemic delivery of MSCs has been described in many studies [23], which demonstrated the homing capacity of MSCs as well as their residence in tissue for extended periods of time, [24]. In this study, safety and efficacy of hAdMSCs was evaluated in immunodeficient mice with various dose levels. A recent study of stem cells in a rat model of middle cerebral artery occlusion reported the greatest therapeutic benefit when a single high cell dose injection was used, rather than multiple infusions of smaller cell doses overall several time points [25]. In the present study, we injected the cultured hAdMSCs into subcutaneous at 2 different concentrations (0/75 × 10^5 or 0/375 × 10^5 cells/ kg). All animals in the high–dose group and low – dose group survived after cell injections, also do not see any side effects in them. Similar results were reported for the systemic administration of allogenic BMMSCs; the IV infusion of 5 × 10^6 MSCs/kg B.W of BMMSCs did not change the overall health or immune status of recipient baboons [26]. Although the safety of intravenous cultured MSCs was confirmed in patients [27] and many human clinical studies of MSCs have been implied to treat diseases such as osteogenesis imperfect [28], metachromatic leukodystrophy [29], acute myocardial infarction [30] and graft – versus – host disease [31], there were some reports presenting that MSCs can induce sarcoma [32] or facilitate the growth of tumor [33]. Therefore, we used subcutaneous injection in the present study. Also the erythrocyte sedimentation rate (ESR) and Quality control (QC) of hAdMSCs was investigated in an animal model. In this ESR test, hAdMSCs were injected into immunodeficient mice and observed for 2 weeks. Even when high doses of cells (0/75 × 10^5 hAdMSCs/kg) and low doses of cells (0/375 × 10^5 hAdMSCs/kg) were injected into mice, side effects were not found in any of the animals.

Conclusion

In the present study, we demonstrated that a subcutaneous route of hAT-MSCs injection can be more effective than an intravenous route to reduce gross and histological signatures of AD in mouse model. These findings led us

to conduct this study using subcutaneous route for the administration of hAT-MSCs. In this study, we also proved that a subcutaneous administration of hAT-MSCs can be successfully used for the treatment of AD and is well tolerated without any safety issues. Therefore, we concluded that the subcutaneous administration of 0/75, 0/375 million cells/kg hAdMSCs appears to be safe and effective as a management option in mouse atopic dermatitis. The efficacy of the treatment was assessed based on the response of each patient compared with its own baseline score before administration. More experiments are needed to evaluate the safety and efficacy of hAdMSCs in human tissues.

Conflict of Interest

The author has no conflict of interest to declare.

Acknowledgements

I thank Dr. Fatemeh. Javani Jouni from Department of Biomedical Engineering, Faculty of Health, Tehran Medical Sciences for differentiation of human Adipose Tissue- mesenchymal stem cells into various cell types.

References

1. Friedenstein, Chailakhjan and Lalykina. "The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells." *Cell Prolif* 3 (1970): 393-403.
2. Erices Alejandro, Paulette Conget and José Minguell. "Mesenchymal progenitor cells in human umbilical cord blood." *Br J Haematol* 109 (2000): 235-242.
3. Yi Zhang, Chang-dong Li, Xiao-xia and Jiang He-lian Li, et al. "Comparison of mesenchymal stem cells from human plcenta and bone marrow." *Chin Med J* (2004)117: 882–887.
4. Zuk Patricia, Zhu, Hiroshi Mizuno and Jerry Huang, et al. "Multilineage cells from human adipose tissue: implications for cell-based therapies." *Tissue Eng* 7 (2001): 211-228.
5. Dominici, Le Blanc, Mueller and Slaper-Cortenbach, et al. "Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement." *Cytotherapy* 8 (2006): 315-317.
6. Pittenger Mark, Alastair Mackay, Stephen Beck and Rama Jaiswal, et al. "Multilineage potential of adult human mesenchymal stem cells." *Science* 284 (1999): 143-147.
7. Sen Anindita, Renee Lea-Currie, Danuta Sujkowska and Dawn Franklin, et al. "Adipogenic potential of human adipose derived stromal cells from multiple donors is heterogeneous." *J Cell Biochem* 81 (2001): 312-319.
8. Jo Jung-Youn, Sung-Keun Kang, In-Su Choi and Jeong-Chan Ra. "Comparison of neural cell differentiation of human adipose mesenchymal stem cells derived from young and old ages." *Dev Reprod* 13 (2009): 227-237.
9. Mouiseddine Francois, Semont, Sache and Allenet, et al. "Human mesenchymal stem cells home specifically to radiation-injured tissues in a non-obese diabetes/severe combined immunodeficiency mouse model." *Br J Radiol* 80 (2007): S49-S55.
10. Vieira, Brandalise, Zucconi and Secco, et al. "Isolation, characterization, and differentiation potential of canine adipose-derived stem cells." *Cell transplant* 19 (2010): 279-289.
11. Torres Fabrício Carvalho, Consuelo Junqueira Rodrigues, Ithamar Nogueira Stocchero and Marcus Castro Ferreira. "Stem cells from the fat tissue of rabbits: an easy-to-find experimental source." *Aesthetic Plast Surg* 31 (2007): 574-578.
12. Vidal Martin, Gail Kilroy, Mandi Lopez and Jill Johnson, et al. "Characterization of equine adipose tissue-derived stromal cells: adipogenic and osteogenic capacity and comparison with bone marrow-derived mesenchymal stromal cells." *Vet Surg* 36 (2007): 613-622.
13. Williams Kellie, Alicia Picou, Sharon Kish and Angelica Giraldo, et al. "Isolation and characterization of porcine adipose tissue-derived adult stem cells." *Cells Tissues Organs* 188 (2008): 251-258.
14. Yamamoto Naoki, Hirohiko Akamatsu, Seiji Hasegawa and Takaaki Yamada, et al. "Isolation of multipotent stem cells from mouse adipose tissue." J Dermatol Sci 48 (2007): 43-52.
15. Zuk Patricia, Min Zhu, Peter Ashjian and Daniel De Ugarte, et al. "Human adipose tissue is a source of multipotent stem cells." *Mol biol cell* 13 (2002): 4279-4295.
16. Wislet-Gendebien Sabine, Pierre Leprince, Gustave Moonen, and Bernard Rogister. "Regulation of neural markers nestin and GFAP expression by cultivated bone marrow stromal cells." *J Cell Sci* 116 (2003): 3295-3302.
17. Bonab Mandana Mohyeddin, Kamran Alimoghaddam, Fatemeh Talebian and Syed Hamid Ghaffari, et al. "Aging of mesenchymal stem cell *in vitro*." *BMC cell biol* 7 (2006): 1-7.
18. Briquet Alexandra, Sophie Dubois, Sandrine Bekaert and Marie Dolhet, et al. "Prolonged ex vivo culture of human bone marrow mesenchymal stem cells influences their supportive activity toward NOD/SCID-repopulating cells and committed progenitor cells of B lymphoid and myeloid lineages." *Haematologica* 95 (2010): 47.
19. Sordi, Valeria. "Mesenchymal stem cell homing capacity." *Transplant* 87 (2009): S42-S45.
20. Gerdoni Ezio, Barbara Gallo, Simona Casazza and Silvia Musio, et al. "Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis." *Ann Neurol* 61 (2007): 219-227.
21. Uccelli Antonio, Vito Pistoia and Lorenzo Moretta. "Mesenchymal stem cells: a new strategy for immunosuppression?" *Trends Immunol* 28 (2007): 219-226.
22. Aggarwal Sudeepta and Mark Pittenger. "Human mesenchymal stem cells modulate allogeneic immune cell responses." *Blood* 105 (2005): 1815-1822.
23. Mackenzie Tippi and Alan Flake. "Human mesenchymal stem cells persist, demonstrate site-specific multipotential differentiation, and are present in sites of wound healing and tissue regeneration after transplantation into fetal sheep." *Blood* 27 (2001): 601-604.
24. Devine Steven, Amelia Bartholomew, Nadim Mahmud and Mary Nelson et al. "Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion." *Exp Hematol* 29 (2001): 244-255.
25. Omori Yoshinori, Osamu Honmou, Kuniaki Harada and Junpei Suzuki et al. "Optimization of a therapeutic protocol for intravenous injection of human mesenchymal stem cells after cerebral ischemia in adult rats." *Brain Res* 1236 (2008): 30-38.
26. Beggs Kirstin, Alex Lyubimov, Jade Borneman and Amelia Bartholomew et al. "Immunologic consequences of multiple, high-dose administration of allogeneic mesenchymal stem cells to baboons." *Cell transplant* 15 (2006): 711-721.
27. Lazarus HillardM, Haynesworth, Gerson and Rosenthal et al. "Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): implications for therapeutic use." *Bone marrow transplant* 16 (1995): 557-564.
28. Horwitz Edwin, Patricia Gordon, Winston Koo and Jeffrey Marx et al. "Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone." *Proc Natl Acad Sci* 99 (2002): 8932-8937.
29. Koc, Day, Nieder and Gerson et al. "Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH)." *Bone marrow transplant* 30 (2002): 215-222.
30. Chen Shao-liang, Wu-wang Fang, Fei Ye and Yu-Hao Liu et al. "Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction." *Am J Cardiol.* 94 (2004): 92-95.
31. Le Blanc Katarina, Francesco Frassoni, Lynne Ball and Franco Locatelli et al. "Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study." *The Lancet* 371 (2008): 1579-1586.
32. Tolar, Abdallah, Schrøder and Kassem et al. "Sarcoma derived from cultured mesenchymal stem cells" *Stem Cells* 25 (2007): 371–379.
33. Zimmerlin Ludovic, Albert Donnenberg, Peter Rubin and Per Basse et al. "Regenerative therapy and cancer: *in vitro* and *in vivo* studies of the interaction between adipose-derived stem cells and breast cancer cells from clinical isolates." *Tissue Engineering* 17 (2011): 93-106.

How to cite this article: Yeganeh Davoudi, Minoo Mahmoodi, Masoud Ghorbani, Mohammad Ali Nilforoushzade, Rahim Ahmadi. "Safety and Efficacy of Subcutaneous Infusion of Human Adipose Tissue-Derived Mesenchymal Stem Cells in the Treatment of Mouse Atopic Dermatitis: A Pilot Study." J Neurol Disord 8 (2021): 290