Assessing biological aging following systemic administration of bFGF-supplemented adipose-derived stem cells with high efficacy in an experimental rat model

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Received April 8, 2018; Accepted August 14, 2018

DOI: 10.3892/etm.2019.7251

Abstract. Biological aging (BA) is a tool for comprehensive assessment of individual health status. A rat model was developed for measuring BA by intravenously administering adipose-derived stem cells (ADSCs) into rats and evaluating several biochemical parameters. In addition, the effect of basic fibroblast growth factor (bFGF) on the differentiation potential of ADSCs was analyzed. A total of 12 male Sprague Dawley rats were divided into autologous ADSC administration (n=6) and saline administration (n=6) groups. The ADSC administration group was further divided into the bFGF supplemented (n=3) and bFGF non-supplemented (n=3) groups. Biochemical parameters and antioxidant potential were evaluated prior to fat harvest and ADSC administration, as well as 1, 3, and 5 weeks following ADSC administration. ADSC administration regulated inflammation, renal and hepatic functions, and levels of antioxidant enzymes. The cell doubling time of the bFGF-supplemented group was shorter (P=0.0001) than that of the bFGF non-supplemented group. Renal and hepatic functions were maintained with bFGF supplementation, which possibly enhanced the effect of ADSCs. The rat model developed in the present study may promote better understanding of BA in the context of bFGF-supplemented ADSC administration.

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Key words: biologic aging, biochemical parameter, adipose-derived stem cell, basic fibroblast growth factor, rat model

Introduction

Human life expectancy has increased to the age of 90 years and according to statistics obtained from the United States, it is estimated that by 2050, the number of people aged 60 or above is expected to more than double from the number in 2017 (1). Owing to the rapid aging of a considerable proportion of society, there is growing interest in improving the quality of life, including understanding the anti-aging effect. Aging, however, has different characteristics, such as progressive aging of specific organs within individuals. Although, it is difficult to objectively judge the extent of aging of an individual, several attempts have been made nonetheless (2). Piantanelli *et al* (3) elaborated on the issue of the correct choice of uncorrelated markers, Jackson *et al* (4) interpreted aging based on its correspondence with individual life expectancy, and Nakamura *et al* (5) used monkeys to study biomarkers of aging. Thus, objective criteria are required for effectively evaluating aging.

The mechanism of aging is complex and associated with various factors. Several theories have been proposed to explain the process of aging, although none are completely satisfactory (6). In particular, DNA damage and cell destruction due to excessive production and accumulation of superoxide free radicals are known to be important factors of aging (7). Studies on antioxidation, such as those on the redox pathway, have been actively conducted to help prevent aging (8-10).

At present, interest in stem cell research and experimentation is increasing. Adipose-derived stem cells (ADSCs) are relatively easy to harvest and can be extracted in large quantities. Thus, their clinical applications are being studied in various fields, such as tissue regeneration and wound healing (11).

ADSCs act by inducing differentiation and releasing cytokines, such as vascular endothelial growth factor, basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), and interleukin-6, which have been reported to exert an anti-aging effect by acting as antioxidants and inducing

angiogenesis (12-14). Therefore, a model for assessing anti-aging effects using ADSCs is required.

Chronological age refers to the degree of aging calculated from the time following birth (10), but it fails to provide an accurate indication of the aging process (15). One of the typical methods for evaluating aging is measurement of biological aging (BA), which represents the overall physiological or functional age of each individual, and individuals born at the same time may have different degrees of BA (16). Therefore, BA is a tool for comprehensive assessment of individual health status, including genetic and environmental factors, and its determination is an objective method for assessing disease status and the degree of functional impairment (17).

Biochemical parameters should be evaluated to examine BA (18). However, to the best of our knowledge, biochemical parameters have not been extensively evaluated in a rat model following ADSC administration for assessing BA. Furthermore, the efficacy of intravenous systemic ADSC administration is still debated (19). Therefore, the aim of the present study was to develop a rat model for measuring BA by evaluating a number of biochemical parameters post-intravenous ADSC administration. In addition, the effect of bFGF on ADSC culture was analyzed by comparing bFGF-supplemented and non-supplemented groups.

Materials and methods

Animal experiments. A total of 12 male 6-week-old Sprague Dawley rats (C57BL/6N; Orient-Bio, Inc., Seongnam, Korea) with mean body weight of ~350 g were used to evaluate the effects of ADSCs. Animals were maintained at a controlled temperature (22±2˚C) and humidity (55±5%) with a 12 h light/dark cycle under specific-pathogen-free conditions with free access to food and water. The rats were housed in an animal facility and treated in accordance with the Guide for the Care and Use of Laboratory Animals of Seoul National University Boramae Hospital. Following a two-week quarantine period, 12 rats were divided into the ADSC administration (experimental group; n=6) and saline administration (control group; n=6) groups. The ADSC administration group was also divided into bFGF supplemented (n=3) or bFGF non-supplemented (n=3) groups.

Harvesting of autologous inguinal fat pads and preparation of ADSCs. All surgical procedures were performed under aseptic conditions. Rats were anesthetized by intraperitoneal administration of a combination of Zoletil (Zoletil 100 at 20 mg/kg; Virbac Korea Co., Ltd., Seoul, Korea) and xylazine (Rompun at 10 mg/kg; Bayer, Shanghai, China). Subsequently, the left inguinal region was shaved and draped, and a 2.0-cm incision was made along the inguinal fold. Then, the autologous inguinal fat pad (~3 g) was harvested.

The isolated fat tissues were washed thrice in PBS. Following rinsing, the fat tissue was digested using 0.2 U/ml collagenase NB6 GMP grade (SERVA Electrophoresis GmbH, Heidelberg, Germany) at 37˚C for 1 h with shaking. Stromal vascular fraction was isolated from the digested fat tissue by centrifugation at 252 x g for 5 min at room temperature and filtering through a 100-μm nylon mesh. The isolated cells were washed twice with PBS and plated in a T75 flask in α-minimal essential medium (α-MEM; WelGENE, Inc., Daegu, Korea). Rat ADSCs (rADSCs) were cultured in α-MEM supplemented with 10% fetal bovine serum (FBS; WelGENE, Inc.), 2 mM L-glutamine, and 100 U/ml penicillin/streptomycin. The flasks were maintained in a tissue culture incubator at 37˚C and 5% carbon dioxide. Cultured cells were harvested following trypsinization when they exceeded 90% confluence and sub-cultured at a density of $3x10^5$ cells/T75 flask up to passage 3. The medium was changed every 2-3 days.

bFGF treatment. Recombinant rat bFGF (3339-FB; R&D Systems, Inc., Minneapolis, MN, USA) was added to the growth medium in the bFGF supplemented group. The bFGF concentration in the medium was 5 ng/ml. rADSCs at passage 0 and 1 were seeded in a T75 flask at an initial density of $3x10^5$ cells and cultured for up to 3 passages. The medium was changed every 2-3 days.

Determination of ADSC doubling time. The doubling time of ADSCs in bFGF supplemented and α-MEM and 10% FBS was calculated via a cell counting method with the following formula: PD=t x Log2/(LogC2-LogC1). PD=Population doubling, t=24 h, Log=10 based Log, C1=1st cell count and C2=2nd cell count.

Identification of ADSC by flow cytometry and differentiation assay. A routine protocol was used for identification of ADSCs. Flow cytometry analysis was performed to analyze the phenotype of cultured rADSCs. Nonspecific sites were then blocked in 2% BSA (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with PBS for 1 h at room temperature. ADSCs at P3/P4 were stained for 2 h at room temperature with antibodies for different cluster of differentiation (CD) antigens, such as CD90-fluorescein isothiocyanate (FITC; 1:50; cat. no. REA897; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), CD44-FITC (1:100; cat. no. L178; BD Biosciences, San Jose, CA, USA), and CD45-FITC (1:100; cat. no. HI30; BD Biosciences) prior to administration. rADSCs were grown until 80% confluence, trypsinized, and pelleted by centrifugation at 252 x g for 5 min at 37˚C. Subsequently, ~$3x10^5$ cells were resuspended in 100 μl FACS buffer (WelGENE, Inc.) containing 0.5% BSA in PBS. For FACS analysis of surface markers, each sample was incubated for 30 min at 4˚C in the dark. Following incubation, the labeled cells were diluted with 1 ml FACS buffer, and pelleted at 252 x g for 5 min at room temperature and resuspended in 500 μl FACS buffer. Then, ~$2x10^4$ cells were analyzed per sample using the BD FACS Accuri flow cytometer (BD Biosciences).

Alizarin red S staining was performed for detecting osteogenesis and oil red O staining for detecting adipogenesis post-ADSC differentiation. Briefly, to explore the potential of isolated ADSCs for osteogenic differentiation, ADSCs were seeded at a density of $1x10^4$ cells/cm^2 in 10-cm culture dishes, and were grown with osteogenic induction medium, which was comprised of Dulbecco's modified Eagle's medium (DMEM; WelGENE, Inc.) supplemented with 0.1 mM dexamethasone, 50 mM ascorbate, and 10 mM β-glycerophosphate sodium. Mineralization of ADSCs was determined by alizarin red S

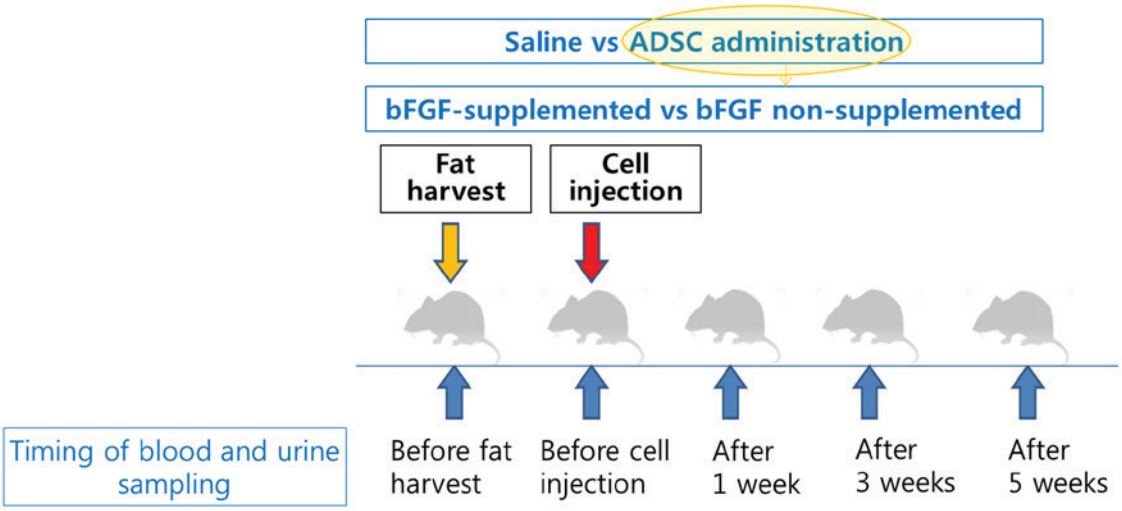

Figure 1. The schematic schedule of the present experimental protocol. The isolated fat tissues were washed and digested. A total of 10^6 ADSCs (P2) were administered via the femoral vein. At fat harvest, ADSC injection, and 1,3, and 5 weeks later, blood and urine samples were analyzed. ADSC, adipose-derived stem cells; bFGF, basic fibroblast growth factor.

(Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) staining on day 21 post-drug treatment, and the staining was visualized and photographed using bright-field microscopy (magnification, x40).

Additionally, adipogenic differentiation was induced by incubating ADSCs in adipogenic induction medium, which contained DMEM supplemented with 10% FBS, 60 μmol/l indomethacin, 0.5 mmol/l 3-isobutyl-1-methylxanthine, 1 μmol/l dexamethasone, 5 μg/ml insulin, and 5 μg/ml gentamicin sulfate (Sigma-Aldrich; Merck KGaA). Following 3 weeks of induction at 37˚C, cells were washed once with PBS and fixed in 4% formaldehyde for 15 min at room temperature. Fixed cells were washed in 60% isopropyl alcohol, and lipid droplets were stained for 1 h at room temperature using the oil red O staining kit (Sigma-Aldrich; Merck KGaA). Following three consecutive washes in deionized water, the stained cells were photographed using bright-field microscopy (magnification, x40; Thermo Fisher Scientific, Inc.).

In addition, chondrogenic differentiation was assessed. Confluent ADSCs were incubated at 37˚C in chondrogenic differentiation medium containing DMEM supplemented with 10% FBS, 0.1 μM dexamethasone, 10 ng/ml TGF-β1 (R&D Systems, Inc., Minneapolis, MN, USA), insulin-transferrin-selenium (Life Technologies; Thermo Fisher Scientific, Inc.), and 50 μg/ml ascorbate at 37˚C. Medium was changed every 2 days for 3 weeks.

Systemic administration of autologous ADSCs. The following surgical procedures were performed ~2 weeks following fat harvesting and completion of autologous ADSC preparation. Following adequate anesthesia, ADSCs (P2, $1x10^6$ cells/300 μl PBS/rat body weight of 300-400 g) were administered to the experimental group and the control group was administered with normal saline via the femoral vein. The blood and urine samples of both groups were analyzed five times (prior to fat harvest and cell administration, and following 1, 3, and 5 weeks (Fig. 1.)

Assessment of BA. Blood and urine samples were used for biochemical investigation. Serum was collected by centrifugation at 13,000 rpm (18,928 x g) for 20 min at 18˚C. Whole blood was collected in an EDTA tube and serum and whole blood was transferred to the Neodin Veterinary Laboratory (Seoul, Korea) for complete blood count (CBC) analysis, which included estimation of white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, red cell distribution width, platelets and mean platelet volume (MPV). Chemical investigations included assessment of albumin, total bilirubin, blood urea nitrogen (BUN), creatinine, uric acid, aspartate transaminase, alanine transaminase (ALT), cholesterol, amylase, calcium, phosphorus, lipase, high density lipoprotein (HDL), and low-density lipoprotein (LDL) levels. Enzyme assays, such as those for superoxide dismutase (SOD; using the Superoxide Dismutase Activity Assay kit; cat. no. ab65354; Abcam, Cambridge, UK) activity, total antioxidant activity (TAC; using the Total Antioxidant Capacity Assay kit; cat. no. ab65329; Abcam), and catalase activity (CT; using the Catalase Assay kit; cat. no. 707002; Cayman Chemical Company, Ann Arbor, MI, USA) were performed according to the manufacturer's instructions.

Urine was collected using metabolic cages. The urine was stored in plastic test tubes at 4˚C prior to analysis following the scheme presented in the Fig. 1. The urine samples were weighed at the time of collection from the cages and transferred to Neodin Veterinary Laboratory for analysis of urine protein, BUN, creatinine and creatinine clearance.

Statistical analysis. To build the prediction models, all parameters were clinically classified into biochemical profiles. Repeated measures of analysis of covariance (RM ANCOVA) were performed to identify the differences between groups of measured values at each time-point. In cases when interactions were significant, additional ANCOVA was performed

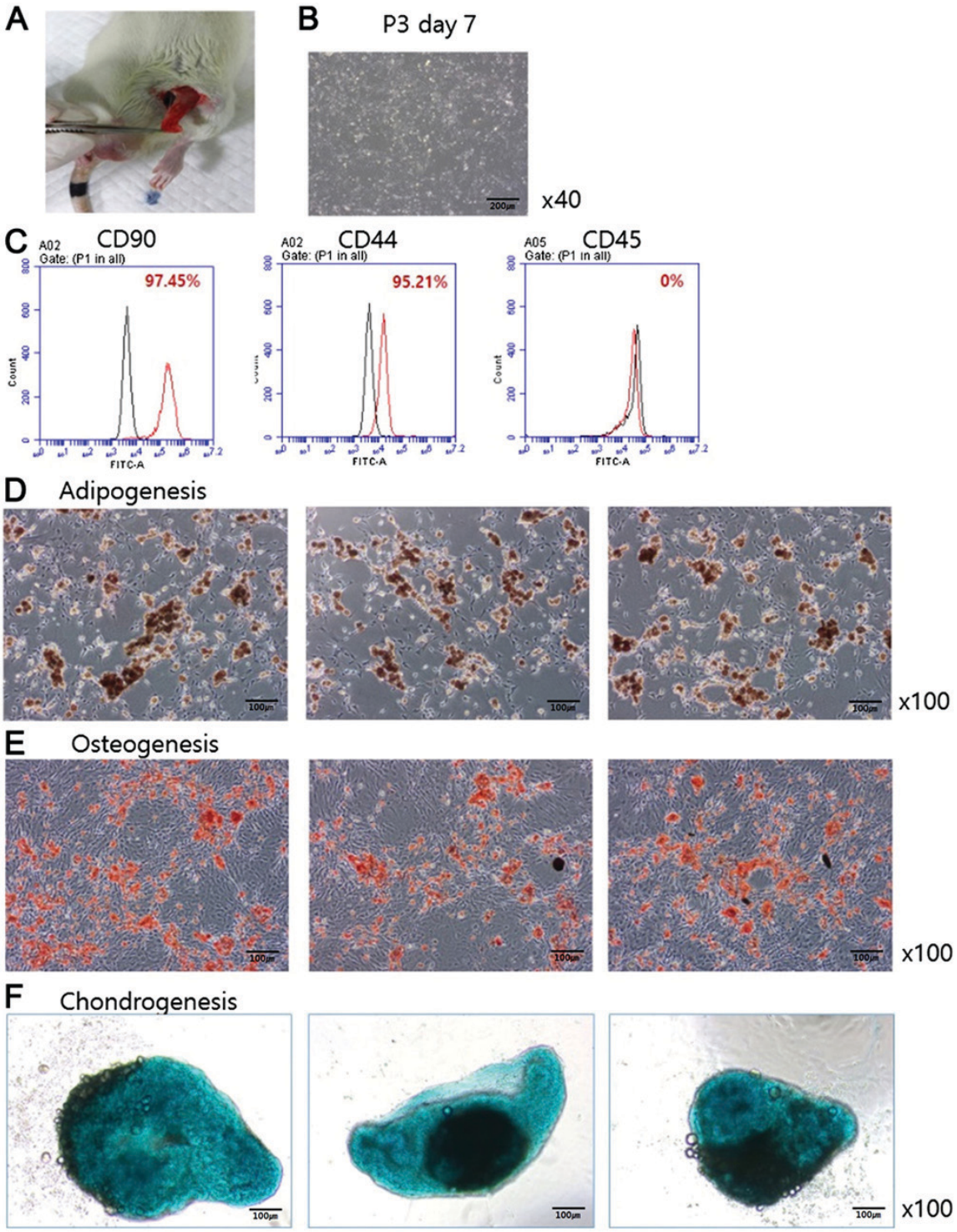

Figure 2. (A) Harvesting autologous inguinal fat pads from each rat. Following shaving and draping of the left inguinal region, a 2.0-cm incision was made along the inguinal fold, and the autologous inguinal fat pads were harvested. (B) Characterization of ADSCs. An optical microscope view of cultured ADSCs was acquired (magnification, x40). (C) Flow cytometry analysis with common stem cell markers CD90 and CD44 and hematopoietic marker CD45 for characterizing autologous ADSCs prior to administration. (D) Alizarin red S stained cells underwent osteogenic differentiation. (E) Oil red O-positive lipid droplets indicate that cells underwent adipogenic differentiation. (F) Alcian blue staining was performed to detect chondrogenic differentiation. ADSC; adipose-derived stem cells; CD, cluster of differentiation; FITC, fluorescein isothiocyanate.

at each time-point. The non-parametric RM ANCOVA was performed following correcting the same value at time 1 as the point prior to cell/saline administration. When the interactions were significant at the significance level of 5%, non-parametric ANCOVA was performed by correcting the values from time 1 to time 5. At this time, the adjusted P-value was obtained using the Sidak method to prevent the increase in Type I errors due to multiple tests (20). Analyses were conducted using SAS version 9.2 (SAS Institute, Inc., Cary, NC, USA). $P<0.05$ was considered to indicate a statistically

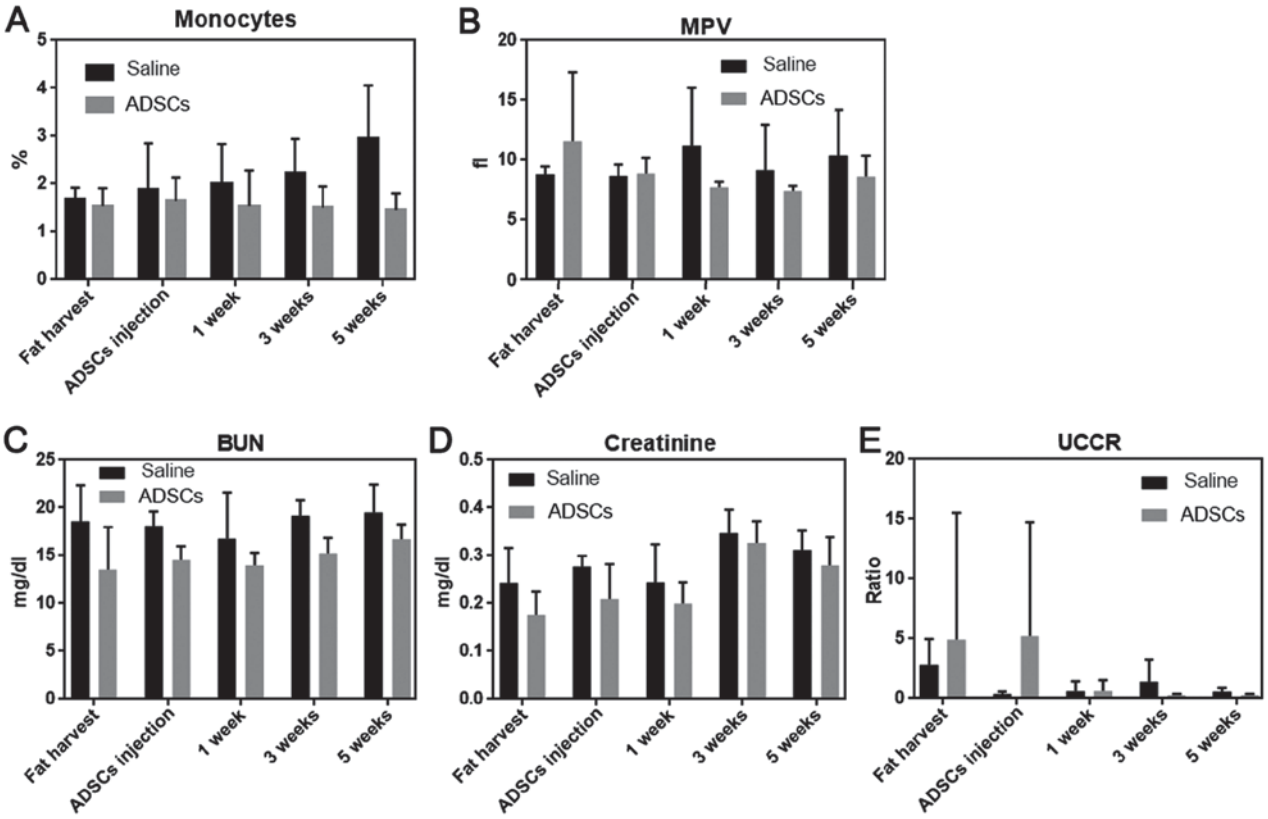

Figure 3. Alterations in leukocyte variables and serum or urine kidney signal. Blood and urine were obtained from the rat model each time-point, and complete blood count and chemical analyses were performed. Measurements in lean control and with ADSC administration were evaluated for (A) blood monocytes, (B) MPV, (C) serum BUN, (D) serum creatinine, and (E) UCCR are presented. The value of monocytes was sustained in ADSC injected group compared with that in saline group, which was increased. Conversely, all others (MPV, BUN, creatinine and UCCR) were downregulated following ADSC administration. Data are presented as the mean ± standard error of the mean. ADSC; adipose-derived stem cells; MPV, mean platelet volume; BUN, blood urea nitrogen; UCCR, urine cortisol:creatinine ratio.

significant result. Results were also expressed using t-test for parametric data.

Results

Characterization of ADSCs and their multilineage differentiation ability. Autologous inguinal fat pad was harvested (Fig. 2A) and ADSCs were cultured, as presented in Fig. 2B. ADSCs exhibited stem cell-like features such as strengthened fibers that could migrate around the cell cluster. Prior to ADSC administration, flow cytometry was used to evaluate the phenotype of ADSCs (Fig. 2C). CD90, the marker for progenitor cells, and CD44, the marker for ADSCs, were present on 97.45 and 95.21% of the cells, respectively, whereas the hematopoietic cell marker CD45 was absent. Furthermore, osteogenic, adipogenic, and chondrogenic differentiation potentials of ADSCs were monitored. Oil red O staining following three weeks of induction under adipogenic conditions indicated that ADSCs underwent adipogenic differentiation (Fig. 2D). Alizarin red S (0.1%) was used to study osteogenic differentiation potential, and results suggested that the majority of ADSCs exhibited osteogenic differentiation potential (Fig. 2E). ADSCs were incubated in chondrogenic differentiation medium containing TGF-β for 3 weeks, and alcian blue staining was performed to examine chondrogenic differentiation of ADSCs (Fig. 2F). Results indicated that ADSCs successfully underwent chondrogenic differentiation.

ADSCs affected aging-related metabolic parameters. Aging is a progressive degenerative process tightly integrated with inflammation. To monitor the inflammatory signals following fat tissue extraction, CBC was monitored using rat blood samples. The number of blood monocytes was lower in the ADSC administration group than in the saline administration group at each time-point. Additionally, the percentage of monocytes in the blood increased in the saline administration group until five weeks following administration (the time of experiment termination), but it was sustained in the ADSC administration group without any significant change [Fig. 3A; P=0.0081 (data not shown)]. MPV level was higher in the ADSC administration group at the fat pad uptake site, but this was reversed following the uptake. MPV level decreased following ADSC administration, but it increased in the saline group (Fig. 3B; P=0.0126), suggesting that administered ADSCs may be differentiated into adipose cells, which can reduce inflammation in rats.

A major function of the kidneys is to remove waste products and excess fluid from the body. Total serum BUN and creatinine levels were markedly different between saline and ADSC administered groups in a time-dependent manner. BUN (Fig. 3C; P=0.0029) and creatinine (Fig. 3D; P=0.0095)

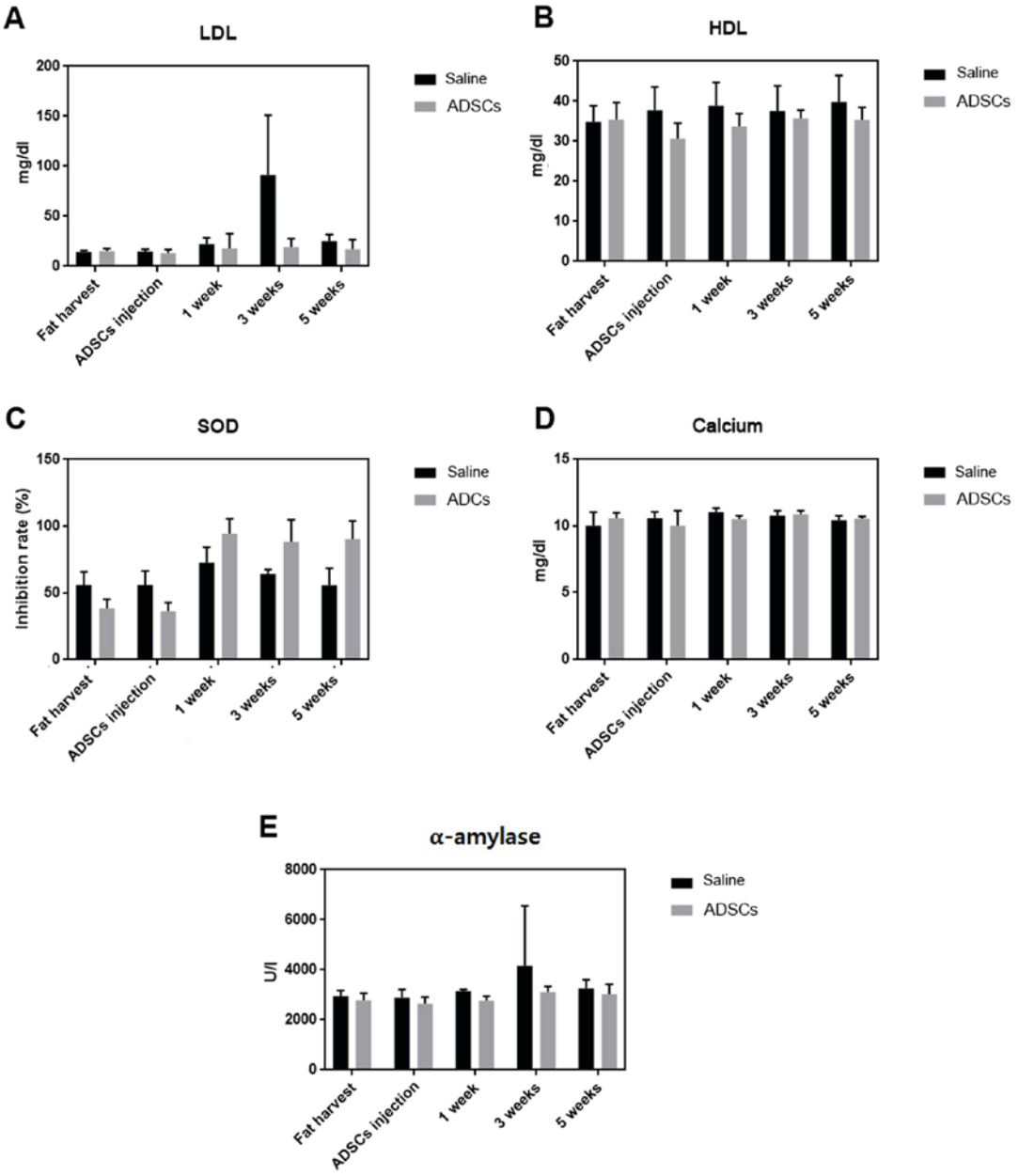

Figure 4. Serum cholesterol, SOD, calcium, and α-amylase levels were determined. Bar graphs presenting (A) LDL, (B) HDL, (C) SOD, (D) calcium and (E) α-amylase levels. Serum LDL and serum HDL levels were downregulated in the ADSC administration group, which tends to reduce the risk of heart diseases. SOD level was increased following ADSC administration. Serum calcium level was lower in the ADSC administration group in a time-dependent manner. The level of α-amylase, a calcium metalloenzyme, was also lower following ADSC administration, thus ADSC can maintain homeostasis of the blood glucose level. Data are presented as the mean ± standard error of the mean. SOD, superoxide dismutase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ADSC; adipose-derived stem cells.

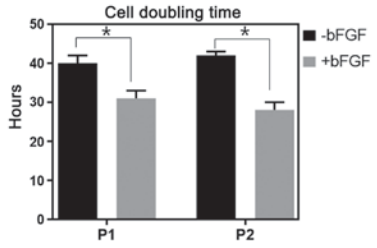

Figure 5. Doubling time of ADSCs cultured with or without bFGF. bFGF markedly increased the cell doubling rate at P1 and P2. Doubling time was markedly shorter in the bFGF treated group, thus indicating the progenitor cell feature. Data are presented as the mean ± standard error of the mean. *P<0.0001. ADSC; adipose-derived stem cells; bFGF, basic fibroblast growth factor; P1, passage 1; P2, passage 2.

levels were reduced following ADSC administration. The urine cortisol:creatinine ratio (UCCR), which detects renal stress due to illness or pre-diabetes mellitus, was also markedly decreased following ADSC administration (Fig. 3E, P=0.0028) (21). Taken together, these findings suggest that ADSCs can maintain normal renal status.

Cholesterol is an essential component of fats that can be metabolized in the liver. LDL deposits on blood vessel walls and adversely affects health. Serum LDL levels were lower in the ADSC administration group than in the saline administration group (Fig. 4A; P=0.0078), and serum HDL level was lower in the ADSC administration group (Fig. 4B; P=0.0069). In the present study, lower HDL levels in the ADSC administration

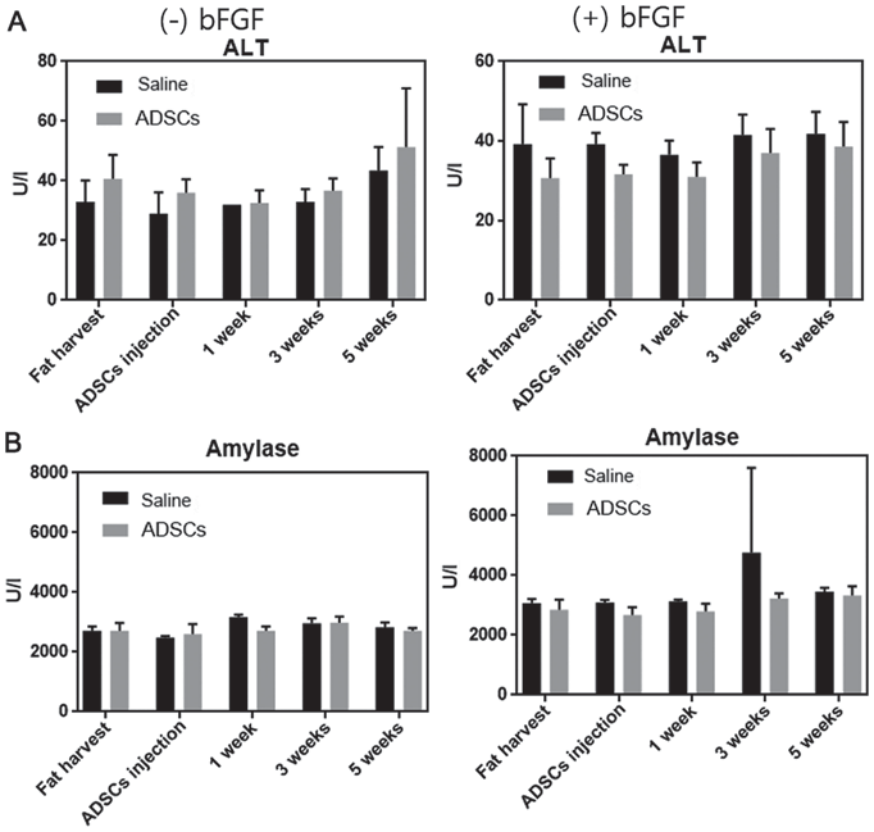

Figure 6. The liver signal can be regulated by administration of ADSCs cultured with bFGF supplementation. (A) ALT and (B) α-amylase levels in bFGF supplemented (right) and non-supplemented (left) groups. ALT level was downregulated in the bFGF-supplemented ADSC administration group. Additionally, α-amylase level was unchanged in bFGF non-treated group, but it was lower in the bFGF-supplemented group. This suggested that ADSCs maintain liver metabolism in anti-aging systems. Data are presented as the mean ± standard error of the mean. ADSC; adipose-derived stem cells; bFGF, basic fibroblast growth factor; ALT, alanine transaminase.

group could be due to lower LDL levels than that observed in the saline administration group.

ADSC administration regulates antioxidant enzyme and calcium and amylase levels. SOD constitutes an important antioxidative line of defense in nearly all living cells exposed to oxygen (22). SOD activity was lower at the sites of fat tissue extraction in the ADSC administration group, but it increased following ADSC administration and was sustained until five weeks following ADSC administration, which was the time of experiment termination (Fig. 4C; P<0.0001).

Total calcium is often measured as a part of routine health screening. Serum total calcium level was different between the control and ADSC administration groups [lower in the ADSC administration group in a time-dependent manner; Fig. 4D; P=0.0002; (data not shown)].

The level of α-amylase, a calcium metalloenzyme that hydrolyzes starch into sugars and contributes to blood glucose regulation, was also determined. The level of α-amylase was lower in the ADSC administration group than in the saline administration group (Fig. 4E; P=0.0213), suggesting that ADSCs can maintain homeostasis of blood glucose level as starch uptake is decreased by α-amylase.

bFGF treatment facilitates stem cell repopulation. bFGF is the growth factor necessary for maintenance of undifferentiated state (23). To compare the effect of bFGF on ADSC culture condition and on the body following ADSC administration, the effect of ADSCs with or without bFGF was studied. bFGF markedly increased the cell doubling rate at passages 1 and 2. The population doubling time was also markedly shorter in the group with bFGF supplementation than that in the bFGF non-supplemented group at passages 1 and 2 (Fig. 5; P<0.001).

bFGF supplementation affects biological markers compared with the non-bFGF supplemented group. Exposure to D-galactose markedly elevated the levels of ALT, which may be an aging marker (24). ALT level increased in the bFGF non-supplemented group, but was lowered in the bFGF-supplemented ADSC administration group (Fig. 6A; P=0.0043). Additionally, α-amylase level was unchanged in the bFGF non-supplemented ADSC administration group, but was lower in the bFGF-supplemented group (Fig. 6B; P=0.0032). These results demonstrated that ADSCs serve a role in the maintenance of liver metabolism in normal and anti-aging systems by decreasing serum ALT and amylase levels. Kidneys are associated with the critical regulation of salt, potassium, and acid content of the body, which are determined by urine protein, BUN, and creatinine levels (Fig. 7). Urine protein level increased in the bFGF non-supplemented group, but decreased in the bFGF-supplemented ADSC administration group (Fig. 7A; P=0.0005). Urine BUN (Fig. 7B; P=0.0003) and creatinine levels (Fig. 7C; P=0.0027) demonstrated a

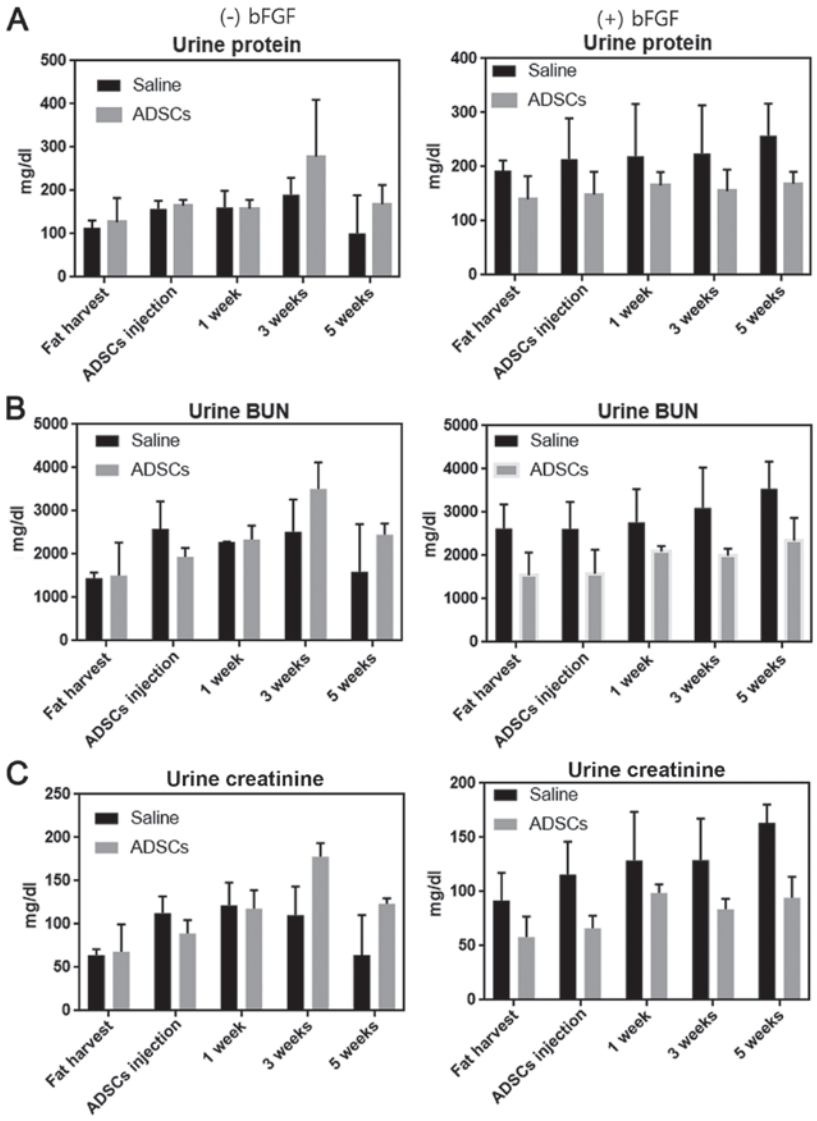

Figure 7. The markers of kidney function changed following ADSC administration in the bFGF supplemented group. (A) Urine protein, (B) urine BUN, and (C) urine creatinine levels in bFGF supplemented (right) and non-supplemented (left) groups. Urine protein was markedly decreased in the bFGF-supplemented ADSC administration group compared with bFGF non-treated group. Urine BUN and creatinine levels exhibited similar patterns to that of urine protein. bFGF affects the biological markers associated with hepatic and renal functions. Data are presented as the mean ± standard error of the mean. ADSC; adipose-derived stem cells; bFGF, basic fibroblast growth factor; BUN, blood urea nitrogen.

pattern similar to that of urine protein. These results demonstrated that bFGF affects the biological markers associated with hepatic and renal functions.

Discussion

Although immortality continues to be beyond our understanding, ways of deterring aging and extending life span are being actively investigated. Owing to these efforts, several aspects of the anti-aging mechanism, particularly the concept of BA, have been identified. Nonetheless, the identification of biomarkers of aging is an ongoing challenge (5). Owing to the various physical, chemical, and environmental factors associated with aging, research on aging has encountered several obstacles. Nevertheless, the ability to quantify aging of an individual numerically and measure the extent of aging precisely would enable physicians to realistically assess the efficacy of a therapy. As a prelude to discussing these approaches and the rationale for their selection, it is appropriate to first evaluate the biological basis of aging.

In 1998, Nakamura *et al* (5) used a monkey model for identifying biomarkers associated with aging, which were divided into three stages, namely those related to longitudinal, cross-sectional and stability analyses. In 2008, Dong *et al* (24) developed equations for evaluating biological age in Korean men and examined whether pathological conditions, such as diabetes, affected BA using this equation.

An experimental model is required to assess BA. To the best of our knowledge, the present study included the first attempt to develop a rat ADSC model using autologous ADSCs to realize this objective. In particular, bFGF was further added to maximize the effect of BA by increasing the differentiation potential of ADSCs. Furthermore, numerous variables and biomarkers of aging were categorized and analyzed. As described below, these physiological variables are considered to represent functions of vital organs associated with successful

aging and maintenance of life (25). For example, low levels or reduction in albumin levels have a significant association with all-cause mortality (26).

Hematology-associated cells undergo coagulation to resolve the vasculopathy associated with oncology (27). The monocytes and MPV levels in the CBC of the ADSC administration group were markedly lower than those of the saline administration group; the low monocyte activity in the cell group could be due to the immunomodulatory effect of ADSCs, and the low MPV rate could represent blood viscosity.

Serum BUN, urine creatinine level and UCCR are indicators of renal function. Routine assessment or screening programs associated with these indicators were used for incidental detection of kidney diseases. Comparison of urine parameters between bFGF-supplemented and non-supplemented groups demonstrated remarkable results. Uric acid level was markedly elevated, whereas urine BUN, protein and creatinine levels were markedly reduced in the bFGF-supplemented group. According to Crasto *et al* (28) renal functional was decreased as concentration of FGF increased. Therefore, development of cell therapy using bFGF and ADSC targeting the kidneys can be beneficial for maintaining nephrological homeostasis.

Cholesterol serves an important role in the liver. LDL assists in transport of metabolites from the liver to regional cells; however, large quantities of LDL are not metabolized properly, which may convert into oxidized cholesterol in blood vessels and cause vasculopathy (29). In the present study, LDL levels in the ADSC administration group was markedly lower than that in the saline administration group. This may be caused by ADSCs, which positively affect the hepatic system and improve BA. Additionally, a positive association between serum LDL levels and the development of the first or subsequent attacks of coronary heart disease was observed in a previous study (30). Furthermore, cholesterol levels decreased markedly in the bFGF non-supplemented group over time, but remained unchanged in the bFGF-supplemented group. Singh *et al* (31) previously reported that FGF induces expression of various cytokines in hepatocytes. Conversely, the ALT level increased in the bFGF non-supplemented group at the late stage, but was maintained at low levels in the bFGF-supplemented group. These results suggest that the hepatic organs associated with ALT are more likely to be affected by bFGF and ADSCs over time.

Serum calcium levels are essential for the evaluation of electrolyte-related metabolism. Calcium levels affect levels of other electrolytes, such as sodium and potassium, through various pumps and channels (32). The serum calcium level was markedly affected by ADSC administration, indicating that ADSCs affect electrolyte metabolism.

The enzyme parameters were compared between the two groups in the present study. Three ELISA kit-based assays were performed for assessing TAC, SOD, and CT activities, which are the major determinants of the rate of aging associated with endogenous oxidative stress. In the present study, SOD activity improved with time following administration of ADSCs, which suggests that ADSCs can be differentiated into damaged cells that produce and activate SOD to lower the aging rate. This has also been confirmed in a worm model and has been identified as an anti-aging target (33). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) serve important roles in the regulation of cell survival. In general, moderate levels of ROS/RNS may function as signals to promote cell proliferation and survival, whereas severe increases in ROS/RNS levels can induce cell death. Under physiological conditions, the balance between generation and elimination of ROS/RNS maintains the proper function of redox-sensitive signaling proteins. Oxidative stress may lead to aberrant cell death and contribute to disease development when redox homeostasis is disturbed (34). The role of ADSCs in these redox signaling pathways should be further studied from a molecular and genetic point of view. Assessment of antioxidant factors in the BA assay is essential considering the anti-aging effects of ADSCs.

It is an important and challenging task to identify a valid biomarker for each affected individual. Experiments with monkey model indicated that valid biomarkers differ between macaques and humans owing to differing environments (5). Furthermore, owing to the requirement of minimum criteria for a potential biomarker (35), identifying a valid biomarker has become a highly sophisticated task.

Taken together, these findings suggest that ADSCs affect the physiological characteristics of an individual. The type of cell therapy that may be applied to the various BA component variables can also be determined. The results of the present study can be used to develop a BA model and customize cell therapy.

However, the present study is not without limitations. First, biopsy could not be performed following tissue sampling and human biopsy was not allowed in a clinical setting. Therefore, blood and urine were sampled. Second, according to the results of the present study, experiments were planned using adult rats. Third, formulating a BA equation for ADSCs was challenging. A number of previous studies have attempted to formulate BA equations using biomarkers (3,24). In the present study, the aim was to develop models for assessing human BA and producing optimized stem cell therapeutics by developing highly efficient stem cell extraction technology from the human adipose tissue. The rat model designed in the present study may form an important basis for further developments in this field.

The present findings suggest that the rat model developed herein may help researchers elucidate the anti-aging mechanism associated with ADSCs along with bFGF supplementation. The present study suggests using a standardized anti-aging rat model for measuring BA with physical and biochemical parameters following intravenous administration of ADSCs.

Acknowledgements

Not applicable

Funding

The present study was supported by grant no. 04-2016-0670 from the SNUH Research Fund and a Mi-Rae international consortium grant (grant no. BR16-0018).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HSB, HYS, HSH and JUP performed the experiments and analyzed the data; YS, SK, HSH and JUP designed and supervised the study; SK, YS and JUP provided crucial input for the project; HSB, HYS and JUP wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Animals used in the present study were housed in a specific pathogen-free animal facility and all experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Seoul Metropolitan Government-Seoul National University, Boramae Medical Center (approval no. 2016-0031).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. World Population Prospects: The 2017 Revision. Executive Summary. United Nations Department of Economic and Social Affairs, Population Division. 2017. https://esa.un.org/unpd/wpp/publications/files/wpp2017_keyfindings.pdf. Accessed June 21, 2017.
2. Klemera P and Doubal B: A new approach to the concept and computation of biological age. Mech Ageing Dev 127: 204-248, 2006.
3. Piantanelli L, Rossolini G, Basso A, Piantanelli A, Malavolta M and Zaia A: Use of mathematical models of survivorship in the study of biomarkers of aging: The role of heterogeneity. Mech Ageing Dev 122: 1461-1475, 2001.
4. Jackson S, Weale MR and Weale RA: Biological age-what is it and can it be measured? Arch Gerontol Geriatr 36: 103-115, 2003.
5. Nakamura E, Lane MA, Roth GS and Ingram DK: A strategy for identifying biomarkers of aging: Further evaluation of hematology and blood chemistry data from a calorie restriction study in rhesus monkeys. Exp Gerontol 33: 421-443, 1998.
6. Davidovic M, Sevo G, Svorcan P, Milosevic DP, Despotovic N and Erceg P: Old age as a privilege of the 'selfish ones.' Aging Dis 1: 139-146, 2010.
7. Liochev SI: Reactive oxygen species and the free radical theory of aging. Free Radic Biol Med 60: 1-4, 2013.
8. Chandrasekaran A, Idelchik MDPS and Melendez JA: Redox control of senescence and age-related disease. Redox Biol 11: 91-102, 2017.
9. Go YM and Jones DP: Redox theory of aging: Implications for health and disease. Clin Sci (Lond) 131: 1669-1688, 2017.
10. Ji LL: Redox signaling in skeletal muscle: Role of aging and exercise. Adv Physiol Educ 39: 352-359, 2015.
11. Mizuno H: Adipose-derived stem cells for tissue repair and regeneration: Ten years of research and a literature review. J Nippon Med Sch 76: 56-66, 2009.
12. Park BS, Jang KA, Sung JH, Park JS, Kwon YH, Kim KJ and Kim WS: Adipose-derived stem cells and their secretory factors as a promising therapy for skin aging. Dermatol Surg 34: 1323-1326, 2008.
13. Bae CY, Kang YG, Kim S, Cho C, Kang HC, Yu BY, Lee SW, Cho KH, Lee DC, Lee K, *et al*: Development of models for predicting biological age (BA) with physical, biochemical, and hormonal parameters. Arch Gerontol Geriatr 47: 253-265, 2008.
14. Fan Y, Jeong JH, You GY, Park JU, Choi TH and Kim S: An experimental model design for photoaging. J Craniofac Surg 26: e467-e471, 2015.
15. Ries W and Pöthig D: Chronological and biological age. Exp Gerontol 19: 211-216, 1984.
16. Finkel D, Whitifield K and Mcgue M: Genetic and environmental influences on functional age: A twin study. J Gerontol B Psychol Sci Soc Sci 50: P104-P113, 1995.
17. Karasik D, Demissie S, Cupples LA and Kiel DP: Disentangling the genetic determinants of human aging: Biological age as an alternative to the use of survival measures. J Gerontol A Biol Sci Med Sci 60: 574-587, 2005.
18. Borkan GA and Norris AH: Biological age in adulthood: Comparison of active and inactive US males. Human Biol 52: 787-802, 1980.
19. Ra JC, Shin IS, Kim SH, Kang SK, Kang BC, Lee HY, Kim YJ, Jo JY, Yoon EJ, Choi HJ and Kwon EN: Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans. Stem Cells Dev 20: 1297-1308, 2011.
20. Kahya M, Wood TA, Sosnoff JJ and Devos H: Increased postural demand is associated with greater cognitive workload in healthy young adults: A pupillometry study. Front Hum Neurosci 12: 288, 2018.
21. Kapoor N, Job V, Jayaseelan L and Rajaratnam S: Spot urine cortisol-creatinine ratio-A useful screening test in the diagnosis of Cushing's syndrome. Indian J Endocrinol Metab 16 (Suppl 2): S376-S377, 2012.
22. ghodaro OM and Akinloye OA: First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J Med 13, 1-7, 2017 (In press).
23. Lotz S, Goderie S, Tokas N, Hirsch SE, Ahmad F, Corneo B, Le S, Banerjee A, Kane RS, Stern JH, *et al*: Sustained levels of FGF2 maintain undifferentiated stem cell cultures with biweekly feeding. PLoS One 8: e56289, 2013.
24. Dong MH, Bettencourt R, Brenner DA, Barrett-Connor E and Loomba R: Serum levels of alanine aminotransferase decrease with age in longitudinal analysis. Clin Gastroenterol Hepatol 10: 285-290.e1, 2012.
25. Park J, Cho B, Kwon H and Lee C: Developing a biological age assessment equation using principal component analysis and clinical biomarkers of aging in Korean men. Arch Gerontol Geriatr 49: 7-12, 2009.
26. Shaper AG, Wannamethee SG and Whincup PH: Serum albumin and risk of stroke, coronary heart disease, and mortatlity: The role of cigarette smoking. J Clin Epidemiol 57: 195-202, 2004.
27. Pinto A, De Filippi R, Frigeri F, Corazzelli G and Normanno N: Aging and the hemopoietic system. Crit Rev Oncol Hematol 48 (Suppl): S3-S12, 2003.
28. Crasto C, Semba RD, Sun K and Ferrucci L: Serum fibroblast growth factor 21 is associated with renal function and chronic kidney disease in community-dwelling adults. J Am Geriatr Soc 60: 792-793, 2012.
29. Kapadia SR, Nissen SE, Ziada KM, Rincon G, Crowe TD, Boparai N, Young JB and Tuzcu EM: Impact of lipid abnormalities in development and progression of transplant coronary disease: A serial intravascular ultrasound study. J Am Coll Cardiol 38: 206-213, 2001.
30. Stone NJ, Bilek S and Rosenbaum S: Recent national cholesterol education program adult treatment panel III update: Adjustments and options. Am J Cardiol 96A: E53-E59, 2005.
31. Singh S, Grabner A, Yanucil C, Schramm K, Czaya B, Krick S, Czaja MJ, Bartz R, Abraham R, Di Marco GS, *et al*: Fibroblast growth factor 23 directly targets hepatocytes to promote inflammation in chronic kidney disease. Kidney Int 90: 985-996, 2016.
32. Joborn H, Lundin L, Hvarfner A, Johansson G, Wide L and Ljunghall S: Serum electrolytes and parathyroid hormone in patients in a coronary care unit. J Intern Med 225: 9-14, 1989.
33. Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Doctrow SR and Lithgow GJ: Extension of life-span with superoxide dismutase/catalase mimetics. Science 289: 1567-1569, 2000.
34. Meo SD, Reed TT, Venditti P and Victor PM: Role of ROS and RNS sources in physiological and pathological conditions. Oxid Med Cell Longev 2016: 1245049, 2016.
35. Sprott RL: Biomarkers of aging and disease: Introduction and definitions. Exp Gerontol 45: 2-4, 2010.