

Investigation of the Reparative and Regenerative Effects of Human Adipose Tissue Mesenchymal Stem Cells on Epidermal Cells Exposed to UVB Ray

UVB Işınına Maruz Kalan Epidermal Hücrelerde İnsan Adipoz Doku Mezenkimal Kök Hücrelerinin Onarıcı ve Canlandırıcı Etkilerinin Araştırılması

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Keywords

Stem cell, keratinocyte, UVB

Anahtar Kelimeler

Kök hücre, keratinosit, UVB

Received/Geliş Tarihi : 29.08.2021

Accepted/Kabul Tarihi : 23.11.2021

doi:10.4274/meandros.galenos.2021.87004

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Abstract

Objective: To investigate the regenerative effects of adipose-derived mesenchymal stem cells (ADMSCs) in immortalized human keratinocyte cells (HaCaT) exposed to ultraviolet B (UVB) rays.

Materials and Methods: Epidermal cells were interacted with UVB rays. Then, treatment was applied to ADMSC mediums prepared in different concentrations. Cells that did not receive UVB light were included in the study as a control.

Results: With the application of human adipose tissue mesenchymal stem cells to HaCaT cells with different concentrations of media prepared, cell viability increased in both cells that received UVB radiation and those that did not. It has been observed that the said increases are more pronounced, especially in cells that have received UVB rays, reaching the maximum value at 100%, which is the highest stem cell medium concentration. ADMSC medium also had an increasing effect on the migration of UVB (+) cells. In UVB (+) cells, decreased levels of myeloid cell leukemia 1 protein (Mcl-1) and phosphorylated extracellular signal-regulated kinases (p-ERK 1/2) and dose-proportional increases were observed after treatment with stem cell medium.

Conclusion: It has been determined that some factors in the ADMSC medium, especially in keratinocytes that have received UVB rays, positively support proliferation and show reparative and rejuvenating functions.

Öz

Amaç: Bu çalışmanın amacı ultraviyole B (UVB) ışınlarının etkisi altında bırakılan immortalize insan keratinosit hücrelerinde (HaCaT), insan adipoz doku mezenkimal kök hücrelerinin (ADMSC) yenileyici etkilerinin araştırılmasıdır.

Gereç ve Yöntemler: Epidermal hücreler, UVB ışınları ile etkileştirildi. Daha sonra farklı derişimlerde hazırlanmış olan ADMSC medyumları ile tedavi uygulandı. UVB ışını almamış olan hücreler, kontrol amacıyla çalışmada yer aldı.

Bulgular: ADMSC farklı konsantrasyonlarda hazırlanmış olan ortam medyumlarının HaCaT hücrelerine uygulanmasıyla birlikte hem UVB ışını alan, hem de UVB ışını almamış olan hücrelerde hücre canlılığının arttığı saptanmıştır. Söz konusu artışların özellikle UVB ışını almış olan hücrelerde daha belirgin düzeyde olduğu, en yüksek kök hücre ortam medyum konsantrasyonu olan %100'lükte maksimum değere

ulaştığı görülmüştür. ADMSC medyumunu, UVB (+) hücrelerin migrasyonunda da artırıcı yönde etki yapmıştır. UVB (+) hücrelerde, myeloid hücre lösemi 1 proteini (Mcl-1) ile fosforillenmiş ekstraselüler sinyalle düzenlenen kinazların (p-ERK 1/2) azalan düzeylerinde, kök hücre ortam medyumunu ile muameleden sonra doz ile doğru orantılı artışlar görülmüştür.

Sonuç: Özellikle UVB ışını almış olan keratinositlerde, ADMSC ortam medyumunda bulunan etmenlerin proliferasyonu olumlu yönde desteklediği, onarıcı ve canlandırıcı fonksiyonlar gösterdiği saptanmıştır.

Introduction

The skin, acts as a protective shield against the environment. Aging is the process of decline in the functions of organismal structures, including the skin. Photoaging is the acceleration of the aging process with the effects of sun rays (1). Photoaging paves the way for the formation of skin cancer with the changes it creates in DNA (2). In photoaging, the formation of deletions in DNA is induced by extracellular matrix degradation (3). Various methods, including autologous tissue fillings, botox injection, and plant compounds, are methods developed to repair the damage caused by ultraviolet rays on human skin (4).

Stem cells can differentiate into various cell types (5). When applied to damaged tissue, they have the ability to repair the damage by the mechanisms of the secreted factors (6). Mesenchymal stem cells are abundant in adipose tissue and easy to obtain from this tissue. It is highly preferred in regenerative medicine can be transplanted safely and effectively to the recipient.

ERKs are serine-protein kinases that are members of mitogen-activated protein kinases (MAPKs) and have crucial functions in a wide variety of cellular events from cell proliferation to apoptosis (7). MAPKs are a signal-carrying cascade system found in all eukaryotes, involved in converting extracellular signals and responses (8).

Myeloid cell leukemia 1 protein (Mcl-1) is a member of the Bcl-2 protein family and has anti-apoptotic properties. It was first detected in differentiating myeloid cells (9). The Mcl-1 protein changes and fluctuates suddenly within the cell in response to external stimuli.

In our study, the effects of adipose-derived mesenchymal stem cells (ADMSC) supernatants on proliferation, migration, and apoptosis in keratinocytes exposed to ultraviolet B (UVB) light were investigated. In order to investigate the regenerative capacity of stem cells, Mcl-1 and p-ERK1/2 analyzes were performed. Our study sheds light on the use

and applicability of ADMSCs in regenerative and reproductive processes.

Materials and Methods

The human immortalized keratinocyte (HaCaT) cells used in our study were obtained from Adnan Menderes University Science and Technology Center laboratory. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum were obtained from Capricorn; keratinocyte growth kit, mesenchymal stem cell basal medium, mesenchymal stem cell growth kit-low serum was obtained from American Type Culture Collection; human phosphorylated extracellular signal-regulated kinases (p-ERK 1/2) Elisa kit, human Mcl-1 Elisa kit were obtained from Elabscience.

Supply of HaCaT Cell Lines and Cell Planting

The cells were grown in growth medium (GM) and were divided again when they reached 80% frequency (Figure 1).

Human Adipose Tissue Stem Cell Recovery

For human adipose tissue stem cells isolated and characterized by flow cytometry according to the methods of Zhu et al. (10) and Francis et al. (11). Cells were started to be isolated from the adipose tissue taken in PBS after plastic surgery. ADMSCs were obtained as a result of applied experiments (Figure 1).

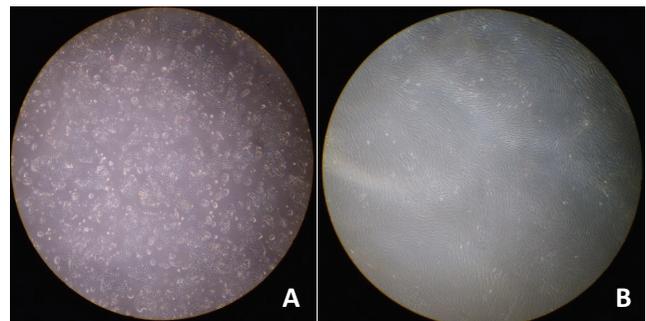


Figure 1. A) When the HaCaT cell reaches 80-90% confluency (x4) (The light microscope image, 5nd passage) B), Human adipose tissue stem cell (x4) (The light microscope image, 2nd passage) HaCaT: Human immortalized keratinocyte

Detection of Surface Antigens in Cells

It has been reported in the literature that it is appropriate to use human ADMSCs up to the 5th passage at most, and the best stem cells are obtained at the passage is between 2-5 (12). For this reason, the cells from which we obtained as the medium in our study were used in the 2th and 3th.

Obtaining Medium for ELISA Studies

HaCaT cells were seeded in GM in a 6-well plate with 125,000 cells per well. After the wells reached 80-90% confluence, were emptied and washed twice with DPBS. The wells were then marked, and 1600 μ L of the relevant medium was added to each well. The medium prepared the day before and containing different concentrations of stem cells (25%, 50%, 75% and 100%) was added to the wells. After 72 hours, medium from the wells were collected. The medium were used in ELISA tests to determine Mcl-1 and p-ERK 1/2 levels.

Obtaining Medium for Experiments from Human Adipose Tissue Stem Cells

ADMSC medium was collected from the supernatant of a high-density ADMSC culture. ADMSC cells were seeded (3th passage) into four separate 25 cm² flasks, and after reaching 80-90% confluency, the cells were washed with DPBS. Then, 6 mL of DMEM + 1% BSA solution was added to the cells, and these cells were incubated at 37 °C and 5% CO₂ for 72 hours. The cells in the flask were collected, centrifuged, and passed through a 0.22 μ m filter again.

Determination of Cell Viability with Microwave Thermoacoustic Tomography Method

It was inoculated into a 96-well plate in 100 μ L of GM DMEM with 10,000 HaCaT cells per well. Phototherapy was applied. ADMSC medium, GM and CM were used to treat cells. All mediums were changed every 72 hours based on the work of Li et al. (1). At the end of this period, the mediums in the wells were aspirated. Microwave thermoacoustic tomography (MTT) solution (prepared as 0.5 mg/mL in serum-free DMEM) was added to the wells as 100 μ L. After waiting 2 hours at 37 °C in a 5% CO₂ incubator (2-4 hours are given in the method), formazan formation becomes visible at the bottom of the wells.

Detecting Apoptosis in Cells

Detection of apoptosis in HaCaT cells was performed with the commercial Biolor ApoPercentage apoptosis kit. HaCaT cells were seeded in a 96-well plate with 30,000 cells per well in 200 μ L of GM. Then

the incubation medium was removed, and one drop DPBS was dropped into the cells. After phototherapy, the cells were aspirated. Cells were incubated for 48 hours. The dye was added to each well, as 5 μ L of apoptotic dye will be in 100 μ L of serum-free medium. After 30 minutes of incubation, the dye was aspirated, the well was washed with DPBS, and pictures were taken from each well for at least 3 areas on an inverted microscope. Then, the absorbance was read at 550 nm after adding 200 μ L dye release agent to the wells and shaking.

Migration Experiment

The cellular migration experiment was performed according to the method described by Liang et al. (13). The cells were seeded in a 6-well plate in 1.5 mL of GM, and the incubation medium was removed to approximately 80-90 occupancy, and the cells were dropped with one drop of DPBS. Cells were taken to phototherapy and exposed to 6 j/cm² dose of UVB light; then, they were brought to the laboratory, and migration analysis was made. The wells were scratched linearly with a 200 μ L pipette tip. Then, incubation was performed with CM and 100% ADMSC medium.

Statistical Analysis

Statistical calculations were performed using International Business Machines SPSS statistical package (version 25). The distribution of numerical data in each group was evaluated with the Shapiro Wilk's test. The Levene test was used to evaluate the distribution of the homogeneity of the groups. In order to compare multiple groups, the F values (an indicator of homogenization of the distribution) or Welch values (an indicator of the inhomogeneity of the distribution) were used for the statistics. While Dunnett T3 method was used for Welch-test, Tukey HSD method was used for F-test in comparison to paired groups. All tests were evaluated according to the value of $\alpha=0.05$. Ethics committee approval was obtained from Aydın Adnan Menderes University Non-Interventional Clinical Research Ethics Committee (decision no: 19, date: 07.02.2019).

Result

MTT Method Results

The degradation of cells that have received UVB can be seen in the Figure 2. Statistically significant differences were found between UVB (-) and UVB (+) values in all groups except the ADMSC medium group with 25% and %50 concentration.

Migration Analysis Results

Images obtained as a result of migration analysis showed that ADMSC medium has the ability to increase migration on HaCaT cells (Figure 3).

Apoptosis Analysis Results

In the UVB (+) group, apoptotic cells after treatment with ADMSC medium are given in Figure 4. The value of

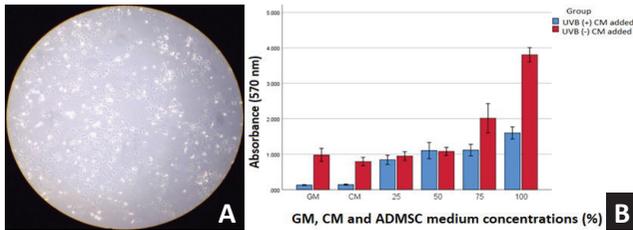


Figure 2. A) Degradation of UVB-received HaCaT cells effect of ADMSC medium on cell viability in UVB (+) and UVB (-) HaCaT cells. GM: Growth medium, CM: Control medium, ADMSC: adipose-derived mesenchymal stem cell, HaCaT: Human immortalized keratinocyte, UVB: Ultraviolet B

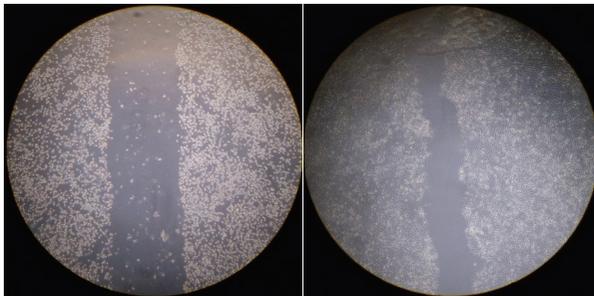


Figure 3. Migration analysis result. After A-control, B-ADMSC medium treatment (48 h) ADMSC: Adipose-derived mesenchymal stem cell

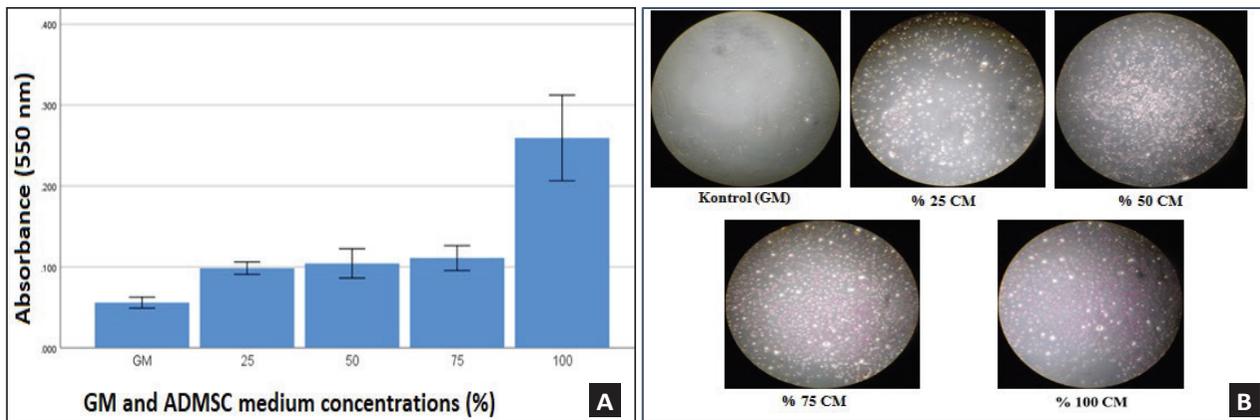


Figure 4. Results of apoptosis in UVB (+) cells after treatment with ADMSC medium A) Graphical representation B), Apoptotic cells under an invert microscope (x4) UVB: Ultraviolet B, ADMSC: Adipose-derived mesenchymal stem cell, GM: Growth medium, CN: Control medium

ADMSC medium with 100% concentration was found to be significantly different from all other groups.

Results of p-ERK 1/2 and Mcl-1 Analysis

In UVB (+) cells, decreased levels of Mcl-1 and p-ERK 1/2, and dose-proportional increases were observed after treatment with stem cell medium. (Figure 5). Both protein levels were found at the highest level in 100% ADMSC medium.

Discussion

Wrinkles in the skin, loss of tightness, hyperpigmentation, epidermal hyperplasia come first among the effects caused by photoaging. Ultimately the formation of skin cancer is among the inevitable consequences of photoaging (14,15). UVB rays coming from the sun directly penetrate the basal cell layer of the skin, and sunburn and stains are formations that accelerate the aging of the skin. With the increase of awareness in this area, people take more care and precautions to protect their skin from sunlight. The use of sunscreen is a precaution for this (16). In addition, some herbs help protect the skin in the long term (17). Compounds such as carnosol have also been accepted to reduce the adverse effects of sunlight on the skin (18). Adipose cells obtained from the abdominal region have been used in the treatment of wrinkles by Zhao et al. (19). Stem cell injection, and somatic cells are among the methods developed and used in skin repair (20). However, more studies are needed in order to apply effective treatment in the treatment of skin damaged by radiation. In this study, the ADMSC medium has been shown to have

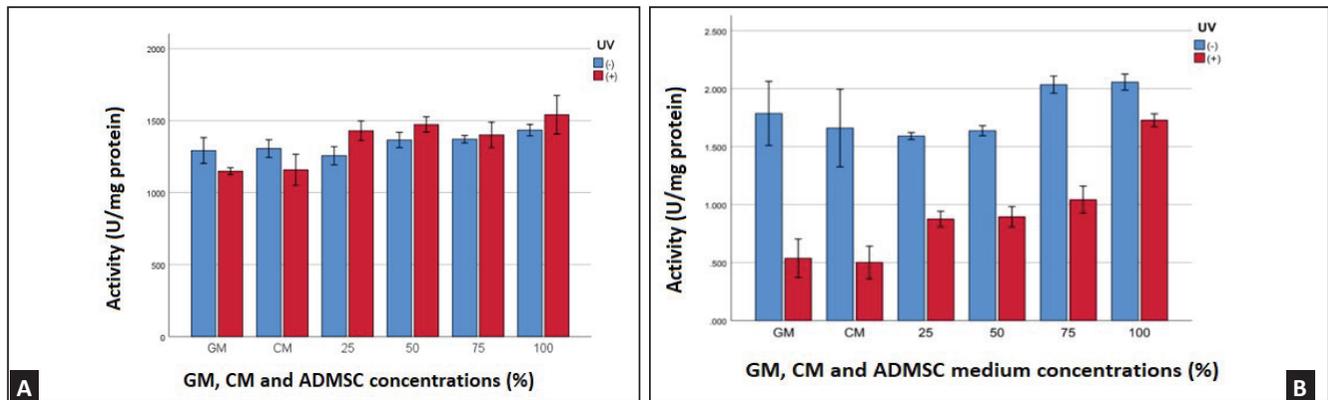


Figure 5. p-ERK 1/2 and Mcl-1 results in UVB (-) and UVB (+) HaCaT cells after treatment with ADMSC medium (A: p-ERK 1/2, B: Mcl-1) p-ERK 1/2: Phosphorylated extracellular signal-regulated kinases, UVB: Ultraviolet B, ADMSC: Adipose-derived mesenchymal stem cell, GM: Growth medium, CM: Control medium

restorative and regenerative effects on keratinocytes exposed to UVB light.

ADSCs are mesenchymal stem cells that can be easily isolated from adipose tissue. Using minimally invasive liposuction, more ADSC can be obtained from other stem cells (21). In previous studies, chorion-derived stem cells (CDSCs) were isolated from the human placenta, and it was shown that they constitute the most abundant source of mesodermal stem cells capable of proliferation and differentiation (22).

In the study conducted by Li et al. (1), it was stated that CDSCs secrete markers specific to mesenchymal stem cells (1). Cell proliferation, cell cycle, and migration results have been shown to support cell division of CDSCs and increase the life span of UVB irradiated keratinocytes. Li et al. (1) found that CDSC medium with 75% concentration showed the best regenerative and restorative effect on photo-aged epidermal cells. In our study, the ADMSC medium with 100% concentration gave the highest result in cell viability. CDSCs secrete active factors and have been shown to stimulate the growth of keratinocyte cells.

CDSCs secrete active factors, and it has been shown to stimulate the growth of keratinocyte cells. The data in our study also support this situation. Li et al. (1) showed that the proliferation rate of UVB (+) cells cultured with GM or CM was significantly reduced compared to UVB (-) cells. In our study, UVB (+) cell viability of HaCaT cells cultured in GM and CM was found to be significantly lower compared to UVB (-).

Li et al. (1) found that the proliferation rates of UVB (+) HaCaT cells cultured in CDSC medium were significantly increased compared to those cultured in CM. Proliferation rates reached the highest value in 75% CDSC medium. At

the same time, it has been shown that stem cell medium increases migration in UVB (+) cells, and the scratch is significantly covered according to UVB (-). Similarly, ADMSC media increased migration in our study. We think that the effect of increasing the migration is valuable for the repair mechanism in cases such as wound healing, especially in sunburns or sun-damaged cells.

In the analysis of apoptosis, although it is thought that treatment with ADMSC medium increases dose-dependent apoptosis in cells, and these findings create a paradox in our study, we think that the cells that underwent apoptosis as a result of phototherapy did not have the opportunity to regenerate, we limited the treatment with medium to 48 hours. In this context, we think that performing this experiment with medium change three times for 72 hours, just like in the cell viability experiment, may affect the results.

The expressions of Mcl-1 and p-ERK 1/2 proteins in UVB (+) HaCaT cells were demonstrated by the western blot method, and it was shown that they decreased, but their expression increased with CDSC medium (1). In our study, it was found that the results of Mcl-1 and p-ERK 1/2 decreased in UVB (+) cells, but increased depending on the dose with ADMSC medium applied in different concentrations.

Zhao et al. (23) demonstrated that epithelial stem cells of human amniotic origin increase the migration and proliferation of keratinocytes. They also showed that the p-ERK 1/2 concentration increased significantly after treatment with HAESC medium. It was shown that the p-ERK 1/2 pathway was inhibited after HaCaT cells were exposed to UVB light in the study conducted by Zhuang et al. (24).

Conclusion

In this study, increases in cell viability and migration were detected in epidermal cells with the application of ADMSC medium. In addition, it was determined that there were increases in p-ERK 1/2 and Mcl-1 values directly proportional to the concentration of ADMSC medium.

It has been determined that the factors in ADMSC medium, especially UVB (+) support the proliferation of keratinocytes, have regenerative and reparative effects. Increasing studies in this field will increase the applicability of ADMSCs in the field of regenerative medicine and expand their usage areas.

Acknowledgment

This study was supported by grants from “Aydın Adnan Menderes University Scientific Research Projects Support Program” (BAP project numbers) TPF-18017.

I would like to thank Ege University Biostatistics and Medical Informatics faculty member Associate Professor Timur KÖSE for his statistical analysis.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from Aydın Adnan Menderes University Non-Interventional Clinical Research Ethics Committee (decision no: 19, date: 07.02.2019).

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Y.T.A., Ç.Y., Design: Y.T.A., Ç.Y., Data Collection or Processing: Y.T.A., Ç.Y., Analysis or Interpretation: Y.T.A., Ç.Y., Literature Search: Y.T.A., Ç.Y., Writing: Y.T.A., Ç.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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