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Effect of glucagon-like peptide-1 on differentiation of adipose derived mesenchymal stem cells into cardiomyocytes

Glukagon benzeri peptit-1'in yağ doku kaynaklı mezenkimal kök hücrelerinin kardiyomiyositlere dönüşmesi üzerindeki etkisi

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ABSTRACT

Aim: Mesenchymal stem cells can easily differentiate into cardiomyocytes in vitro conditions using various protocols. However, the agents used in these protocols have been reported to have some adverse effects on cell viability. Azacitidine is used to differentiate mesenchymal stem cells into cardiac muscle cells. The aim of the present study was to investigate the effects of Exenatide a GLP-1 receptor agonist, on differentiation and viability of human adipose tissue derived stem cells into cardiomyocytes.

Materials and Methods: The effects of Azacytidine and Exenatide on cell viability and proliferation of human adipose tissue derived stem cells were analyzed with cytotoxicity assay. For differentiation procedure, of human adipose tissue derived stem cells were incubated with Azacytidine and Exenatide through four weeks. The morphological alterations of human adipose tissue derived stem cells were monitored and the expressions of cardiomyogenic differentiation markers (cTnI, GATA4 ve MYH7) were evaluated immunohistochemically. Also, cardiac troponin I (cTnI) levels in the cultures were measured using enzyme-linked immunosorbent assay. Results were evaluated by one way analysis of variance (ANOVA) and post-hoc test.

Results: Treatment of the human adipose tissue derived stem cells with Azacytidine significantly decreased cell viability (54.4%) compared to control whereas treatment of cells with Azacytidine + Exenatide prevented cell death in a dose-dependent manner. Cells treated with Azacytidine and Exenatide showed significant morphological alterations consistent with cardiyomyogenic differentiation, and increase in expression cardiomyogenic markers. cTnI levels were found significantly higher in cultures treated separately and together with Azacytidine and Exenatide compared to control.

Conclusion: Overall, these findings suggested that GLP-1 receptor agonist Exenatide may have beneficial effects on cardiomyogenic differention of human adipose tissue derived stem cells by reducing cell damage caused by Azacytidine.

Keywords: Adipose tissue derived mesenchymal stem cell, cardiomyocyte, GLP-1

ÖZ

Amaç: *Mezenkimal kök hücreler, çeşitli protokoller kullanılarak in vitro koşullarda kolaylıkla kardiyomiyositlere farklılaşabilir. Ancak bu protokollerde kullanılan ajanların hücre canlılığı üzerinde bazı olumsuz etkileri olduğu bildirilmiştir.*

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Azasitidin mezenkimal kök hücreleri kalp kası hücrelerine farklandırmak için kullanılmaktadır. Bu çalışmanın amacı, bir GLP-1 reseptör agonisti olan Eksenatid'in insan yağ dokusu kaynaklı kök hücrelerinin kardiyomiyositlere farklılaşması ve canlılığı üzerindeki etkilerini araştırmaktır.

Gereç ve Yöntem: *Azasitidin ve Eksenatid'in insan yağ doku kaynaklı mezenkimal kök hücreler üzerinde hücre canlılığı ve proliferasyonu üzerindeki etkileri ile sitotoksisite testleri yapıldı. Farklılanma protokolü için, hücreler dört hafta boyunca Azasitidin ve Eksenatid ile inkübe edildi. Hücrelerin morfolojik değişiklikleri izlendi ve kardiyomiyojenik farklılaşma belirteçlerinin (cTnI, GATA4 ve MYH7) ekspresyonları immünohistokimyasal olarak değerlendirildi. Ayrıca kültürlerdeki kardiyak troponin I (cTnI) seviyeleri enzime bağlı immünosorbent testi kullanılarak ölçüldü. Veriler, tek yönlü varyans analizi (ANOVA) ve post-hoc testi ile değerlendirildi.*

Bulgular: *İnsan yağ doku kaynaklı mezenkimal kök hücreler üzerine Azasitidin uygulaması, kontrole grubuna kıyasla hücre canlılığını önemli ölçüde azaltırken (%54.4) hücrelerin Azasitidin+Eksenatid ile uygulaması doza bağlı bir şekilde hücre ölümünü önledi. Azasitidin ve Eksenatid uygulanan hücreler, kardiyomiyojenik farklılaşma ile uyumlu önemli morfolojik değişiklikler ve kardiyomiyojenik belirteçlerde artış gösterdi. Ayrı ayrı ve birlikte uygulama yapılan gruplarda cTnI seviyeleri kontrole göre anlamlı derecede yüksek bulundu.*

Sonuç: *Bu bulgular GLP-1 reseptör agonisti Eksenatid'in, Azasitidin uygulamasının neden olduğu hücre hasarını azaltarak İnsan yağ doku kaynaklı mezenkimal kök hücrelerin kardiyomiyojenik farklılaşması üzerinde faydalı etkileri olabileceğini düşündürmektedir.*

Anahtar Sözcükler: *Yağ doku kaynaklı mezenkimal kök hücre, kardiyomiyosit, GLP-1.*

INTRODUCTION

Ischemic cardiovascular disease causes the deaths of approximately 7 million people worldwide per year, and this number is increasing every year (WHO, 2019). Although the current treatments reduce the symptoms due to failure in patients, they are insufficient to regenerate the lost functional heart tissue. In addition to medical and surgical approaches to prevent and treat damage, cellular therapies are becoming increasingly important. Although cellular therapies continue in the experimental field and with a limited number of clinical applications, difficulties in producing functional heart muscle cells for use in applications pose an obstacle to both processes (1-4).

Mesenchymal stem cells (MSCs) were first isolated from the bone marrow and defined as spindle-shaped cells that proliferate and form colonies that adhere to the plastic surfaces (5). In subsequent studies, it has been shown that these cells can transform into osteocytes, adipocytes, and chondrocytes under appropriate conditions (6). MSCs can be isolated from several tissues such as bone marrow, adipose tissue, peripheral blood, amniotic fluid, and umbilical cord (7-10).

Previous studies have reported that MSCs have the capability of differentiation into cardiomyocytes when they have been induced by certain agents such as IL-1 β, TGF-β, ascorbic acid, retinoic acid, angiotensin II and 5-azacitidine (5-aza) (11-15). Of these agents, 5-aza was first synthesized in 1964 by Sorm et al. as a nucleoside antimetabolite and tested in mice with acute myeloid leukemia (16). In later studies, it has been observed that it participates in DNA and RNA structure, causes chromosome damage (17), and has mutagenic properties (18). In an earlier study, 5-aza used to stimulate differentiation of MSCs into beating cardiomyocyttes (19), and its cardiomyogenic activity has also been demonstrated in subsequent studies (11, 14, 20). However, although 5-AzaC adapts the cardiomyogenic transformation, it can cause toxic effects on cell viability. On the other hand, although different methods are used in the transformation of stem cells into heart muscle cells, obtaining functional cardiomyocytes is a challenging process.

The main reason for this is that different cell types interact with each other during the embryological process and these cells also change over time. Human adipose tissue derived mesenchymal stem cells (hATDSCs), which are easier to obtain, can be transformed into several cell types and cardiomyocytes in vitro with various protocols (11-15). However, some conflicting results have been reported regarding the different effects of the agents used in these protocols on cell viability and proliferation.

The incretin hormone glucagon-like peptide 1 (GLP-1) is release from intestinal L cells and accelerates insulin secretion from pancreas

under hyperglycemic conditions (21). Because of these effects, several GLP-1 analogues, including exenatide (Exe) and liraglutide, are currently used for the treatment of type 2 diabetes (22, 23). In addition to its anti-glycemic effects, GLP-1, which has been shown to have receptors in the central nervous system and different tissues such as heart tissue, has also been shown to have protective and apoptosis-reducing effects on nerve cells and cardiomyocytes. In an experimental study conducted by During et al., GLP-1 and its analogs (exendin1-9) were found to increase associative and spatial learning and memory in mice, and it was reported to have protective effects on nerve cells (24). Regarding its effects on the heart tissue, it has been demonstrated that exendin-4, a GLP-1 agonist, could improve the survival of ADSCs and contribute to myocardial repairs after infarction via STAT3 activation (25). Also, it exerts protective effects on cardiac muscle cells against apoptotic cell death triggered by superoxide radicals, which are held responsible for ischemic heart damage (26).

It has been reported that exendin-4 is effective in maintaining the pluripotency feature in human embryonic stem cells and causes a decrease in cell apoptosis. Exendin-4, together with activin A, plays a role in the transformation of human embryonic stem cells into permanent endoderma (27). In addition to studies on the effects of GLP-1 on diabetes and pancreatic ß-cells, its effects on the differentiation of MSCs into osteoblasts and adipocytes have also been investigated in various studies. For example, Lee et al showed that GLP-1 stimulates osteoblastic differentiation of ADSCs, whereas it inhibits adipocyte differentiation. Also, they indicated that ERK (extracellular signal-regulated kinase signaling) pathway seems to be involved in these differentiation processes mediated by GLP-1 (28).

Based on the knowledge in the literature, this study was conducted to investigate whether the GLP-1 analog exenatide has a positive effect on cell viability, proliferation and cardiomyogenic differentiation of hADSCs.

MATERIALS and METHODS

Chemicals

Human adipose tissue derived stem cells (StemPro™ Human Adipose-Derived Stem Cell Kit) were obtained from Thermo Fisher Scientific, USA. All reagents used in experiments were of analytical grade and purchased from Sigma Aldrich GmbH, Sternheim, Germany. Exenatide was purchased from AstraZeneca, The Research-Based Biopharmaceutical Company, Istanbul, Türkiye.

Cell cultures

In this study, commercially purchased human adipose tissue derived mesenchymal stem cell line was grown in culture medium prepared with DMEM. Cells were cultured in horizontal 75 cm^2 filter-capped flasks and incubated at 37^oC in an incubator containing 95% humidity and 5% CO_2 until the cells became confluent. An inverted light microscope was used to observe the vitality and proliferation of the cells. Cells were passaged so that their number per milliliter remained in the range of 10^5-10^6 depending on their growth rate, and medium changes were made regularly every 2-3 days.

Cell viability assay

A cytotoxicity study was conducted to examine the effects of 5-AzaC and Exe on cell viability. For this purpose, the cells were seeded into 96 plates, 10^4 in each well, and only DMEM was applied to the cells in the control group, while 5-AzaC (10 µM) and Exe (10, 100 and 250 nM) in DMEM were applied to the other groups. After the cells were incubated at 37 ^oC for 24 hours, cell viability was determined by the Cell Counting Kit 8 test (29). The principle of this test is based on the conversion of WST8 [2- (2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium, monosodium salt], a water-soluble tetrazolium compound, into a water-soluble formazan compound by dehydrogenase enzymes in cells. The amount of yellow colored formazan formed in this test increases depending on the number of cells. Ready-to-use 10-µl CCK8 solution was added to each well for the test and the cells were taken into the incubator and incubated at 37^oC for 2 hours. The color change that occurred at the end of the period was read in a microplate reader at 450 nm. Cell viability was calculated according to the formula (control %) given below.

Cell viability (%) = (Optical $density_{treatment}$/Optical $density_{control}$) × 100

Cardiomyogenic differentiation of hATDSc

Cardiomyogenic differentiation experiments of hADSCs were performed in accordance with the following protocol (11). Cells were seeded into

12-plate plates at $2x10^4$ per well and incubated in DMEM for the first 48 hours. At the end of 48 hours, the plates were divided into 4 groups as control, Exenatide (Exe), Azacytidine (5-aza) and Exe + 5-aza. Azacitidine (10 µM) and Exenatide (100 nM) solutions were freshly prepared in DMEM. 5-aza treatment was carried out for 24 hours. At the end of the period, the 5-aza-added medium was removed, and the cells were washed twice with PBS, and then DMEM was added to the cells. 5-AzaC treatment was carried out 4 times with 7 days intervals. Exe treatment was applied in DMEM continuously for 4 weeks. Morphological changes in the cells were examined and photographed with a phase contrast microscope for 4 weeks. At the end of experiments, the media were removed, cells were washed with PBS and cTnI measurements were accomplished in cell lysates.

Cardiac troponin I (cTnI) measurement

For the measurement of cardiac troponin I (cTnI), firstly the cells were treated with a cell lysis buffer for 5-10 minutes. Cells whose membranes were lysed were taken from culture plates into centrifuge tubes and centrifuged at 3000 rpm for 5 minutes. At the end of the period, the supernatants in the tubes were taken into eppendorph tubes and stored at -80^oC until the working day. Measurement of cardiac troponin-I (cTnI) levels in cells undergoing differentiation was performed with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Wuhan Fine Biotech Co., Ltd.) according to the manufacturers' instructions. The optical density of the resulting color was read in a microplate reader (Thermo Scientific® Multiskan Go) at 450 nm and interpreted according to the standard calibration curve (30).

Immunohistochemical evaluation of cardiomyogenic biomarkers

Expression of cardiac specific markers such as cTnI (cardiac troponin I), GATA4 (GATA binding protein 4) and MYH7 (myosin heavy chain 7, beta-myosin heavy chain) were evaluated immunohistochemically. For immunohistochemical determination of cardiomyogenic markers, cells ($4x10^4$ in each well) were plated on coverslips coated with poly-L-lysine (PLL). Cells were checked after 24 hours, and differentiation experiments were performed through 4 weeks by adding 5-aza and Exe to the medium. At the end of the differentiation period, medium of the cells was taken and the cells were washed twice with PBS. For fixation of cells, 1 ml of 10% formalin was added to all wells and left for 20 minutes. Then, formalin was removed, and cells were washed twice with PBS. Finally, 1 ml of PBS was added to the cells and prepared for immunohistochemistry procedures. The antibodies used in this study were: anti-CTnI (Finetest FMab09781, 1:100 dilution), anti-GATA4 (Finetest FMab03363, 1:100 dilution), anti-MHY7 (Finetest FMab05478, 1:100 dilution). Following fixation procedure, the fixative was removed, and non-specific binding was blocked by using 1% bovine serum albumin. The suitable primary antibodies against cTnI, GATA4 and MYH7 were added for 45 min at room temperature. After washing with PBS, cells were incubated with biotinylated goat anti-mouse IgG as secondary antibody. Then horseradish peroxidase (HRP) conjugated with Avidin Biotin Complex (Vectastain ABC Kit, Vector Laboratories Inc,) was applied as detection reagent, and finally DAB substrates for peroxidase were used to visualize the antibody binding. All samples were washed in PBS and photographed with an Olympus C-5050 digital camera mounted on an Olympus BX51 microscope (31).

Statistical Analyses

The data obtained in the study were given as mean ± standard error (SEM). SPSS 22.0 (SPSS Inc., Chicago, IL, USA) statistical package program was used for statistical analysis of the data. One-way analysis of variance (ANOVA) was used in the evaluation of the data and the post-hoc Tukey HSD test was used for comparisons between groups, $p<0.05$ was considered statistically significant.

RESULTS

Morphological evaluation of hATDSCs

The growth of the human ADSCs was examined and photographed with an inverted light microscope every day. After cultivation, the round-looking cells adhered to the ground within a few hours and showed a fibroblastic appearance, proliferating rapidly and became ready to passage within 3-4 days was observed (Figure-1).

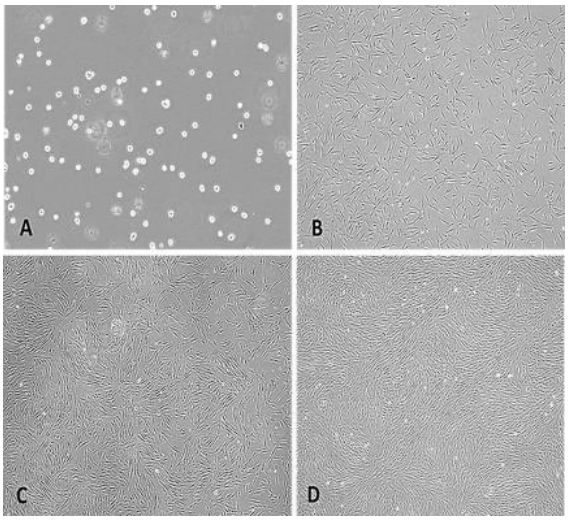

Figure-1. Proliferation of hATDSCs. ***A***. P1/ day 0 ***B.*** P1/ day 1 ***C.*** P1/ day 2 ***D.*** P1/ day 3.

Cell viability Tests

The effects of 5-aza and Exe on mesenchymal stem cells were investigated by cytotoxicity test. 10, 100 and 250 nM Exe applied to cell cultures did not have a negative effect on cell viability. Although it was not statistically significant, a slight increase in cell proliferation was observed especially in the 100 and 250 nM added groups. On the other hand, 5-aza (10 μM) used for cardiomyogenic differentiation reduced cell viability to 54.44% compared to control ($p<0.00005$). It was observed that the addition of Exe (10, 100 and 250 nM) with 5-aza to the medium provided improvement in cell viability depending on the dose (61.4%, 74.31%, 85.55%, respectively). Especially in the groups where 100 nM and 250 nM Exe were added, a significant increase in cell viability was observed compared to the group with only 5-aza ($p <0.005$ and $p <0.0005$, respectively), (Figure-2).

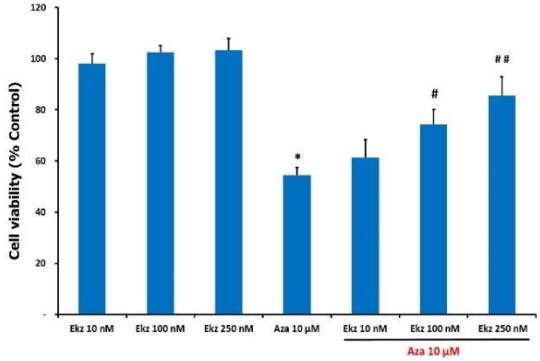

Figure-2. Effects of Exe and 5-aza on viability of hATDSCs.
*p <0.00005 vs. control group
#p <0.005 and ## p <0.0005 vs. 5-aza group

Evaluation of cardiomyogenic differentiation of hATDSCs

Cultures treated with 5-aza and 100 nM Exe for cardiomyogenic differentiation were monitored for 4 weeks. At the end of the first week, it was observed that the cells in the control and Exe applied cultures proliferate and cover the culture dish and have a fibroblast-like appearance. In the 5-aza-treated groups, in addition to fibroblast-like cells, larger and longer "rod" or "sphere" shaped cells were observed compared to the cells in the control group. In the second week of the cultures, the cells in the control and 100 nM Exe-treated groups proliferated and completely covered the culture dish, and the number of rods and spherical cells increased in the 5-aza - applied cultures. It was observed that cells in 5-aza-treated groups formed a syncytium by making cytoplasmic connections with each other at the 3rd week of cultures. At the 4th week of cardiomyogenic differentiation, the presence of cells with 2 and 3 nuclei and myotubule-like formations was noticeable (Figure-3).

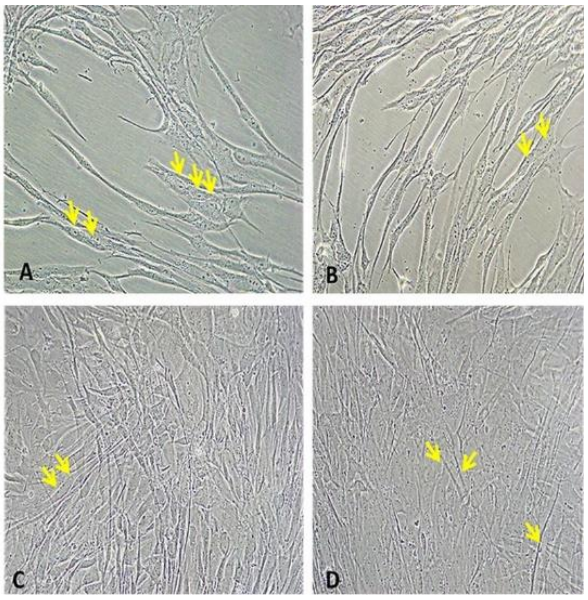

Figure-3. Morphological alterations in hATDSCs treated with 5-aza and Exe.
A. Control, B. Exe, C. 5-aza, D. Exe + 5-aza. Multi-nucleated (2 or 3) cells are seen in C and D, and myotubule-like formations are seen in D (A and B x20, C and D x40 magnification).

Evaluation of cardiac troponin I (cTnI) levels

Cardiac troponin-I (cTnI) levels were evaluated by ELISA in cell lysates. Statistical analysis of the data with one-way ANOVA revealed significant

differences between the study groups. cTnI levels were significantly higher in the groups in which Exe, 5-aza and Exe + 5-aza added compared to the control ($p<0.05$, $p<0.005$, $p<0.0005$, respectively). Also, cTnI level was significantly increased in Exe + 5-aza group than the group given Exe alone ($p<0.0005$). However, no statistically significant difference was observed between the 5-aza and Exe + 5-aza groups (Figure-4).

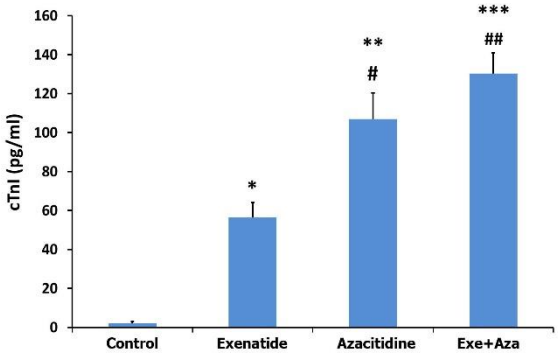

Figure.-4. cTnI levels (pg/ml) in study groups. *p<0.05, ** p<0.005, *** p<0.0005 vs. control group #p<0.005, ## p<0.0005 vs. Exe group

Evaluation of cardiomyogenic biomarkers

Specific markers of cardiomyogenic differentiation such as cTnI, GATA4 and MYH7 were investigated by immunohistochemical staining. In the control group, cell populations demonstrated typical mesenchymal stem cell morphology with symmetrically located nuclei. No staining was detected for anti-cTnI, anti-GATA4 and anti-MYH7 primary antibodies in the control group (Figures 5-7).

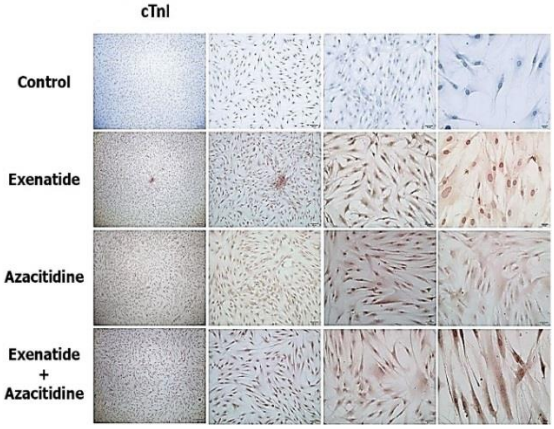

Figure-5. CTnI expression in hATDSCs undergoing cardiomyogenic differentiation (x10, x20, x40, x100 magnification respectively in each group).

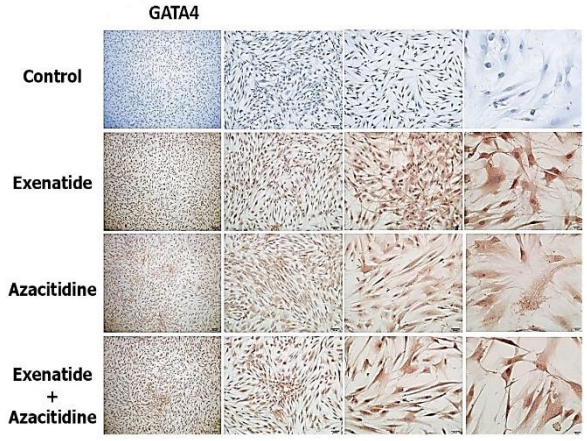

Figure-6. MYH7 expression in hATDSCs undergoing cardiomyogenic differentiation (x10, x20, x40, x100 magnification in each group, respectively).

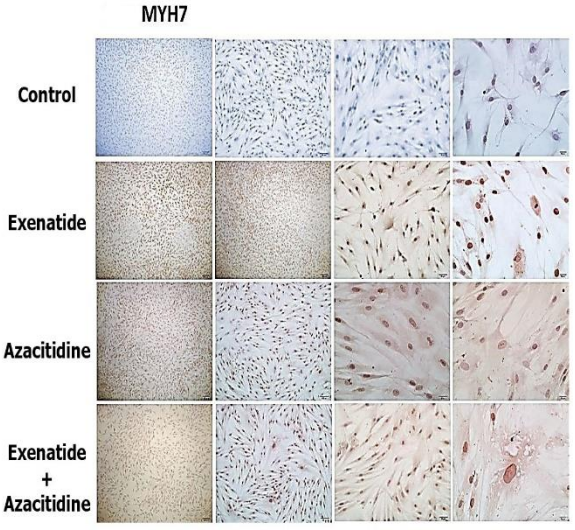

Figure-7. GATA4 expression in hATDSCs undergoing cardiomyogenic differentiation (x10, x20, x40, x100 magnification in each group, respectively).

In the Exe group, it was observed that the proliferation was high in mesenchymal cells, and they showed positive immunostaining for cTnI, GATA4 and MYH7. It was also observed that some cells belonging to this group differ morphologically and have a hypertrophic appearance. In the 5-aza group, it was observed that the cells had the rosette-shaped structures in places. In larger magnifications, it was noticed that the cells were hypertrophic in the central parts of these rosette-shaped structures, unlike the cells in the Exe group, and other mesenchymal cells surrounded these rosette

structures. All samples belonging to this group showed a more positive immunoreaction in terms of anti-cTnI, anti-GATA4 and anti-MYH7 compared to the Exe group (Figures 5-7). Cells in the 5-aza+Exe group, like the 5-aza group, were in the form of rosettes. Also, it was observed that the cells with hypertrophic appearance in the central parts of the different rosette-shaped structures (Figure-7) and were thicker in volume than the cells in all other groups. It was determined that the cells tended to come next to each other, and that cell membrane fusion began to appear in patches (Figure-5). A higher positive immune reaction was observed in all samples belonging to this group for anti-cTnI, anti-GATA4 and anti-MYH7 compared to other experimental groups (Figures 5-7).

DISCUSSION

Until about twenty years ago, it was thought that cardiomyocytes do not have the ability to renew themselves and that the proliferation ability of cardiac tissue is limited to endothelium, smooth muscle cells and fibroblasts (32). In subsequent studies, cells with stem cell characteristics, including more than one type, were found in cardiac tissue (33, 34). However, despite the detection of stem cells in the heart tissue, the reasons for their limited proliferation in pathological conditions such as ischemia with cardiomyocyte loss have not been clarified yet.

Today, the main objectives of cardiac regenerative therapies are to replace the lost functional heart tissue, to trigger the ischemic region to regenerate angiogenesis, to reduce cardiomyocyte loss by preventing cell death, and thus to prevent scar development by preserving the contractility of the heart (35). The low spontaneous recovery capacity of the heart tissue is one of the obstacles in the regeneration of damaged cardiac tissue. Considering the advantages and disadvantages of other cell types, MSCs appear to be one of the most promising options for cardiac regenerative therapies. Among the reasons for preference are that they can be obtained easily as autologous, they can be produced more easily in culture conditions compared to other cell types, and they do not create immune rejection (1-4).

Different methods such as growth factors, co-culture method, and synthetic inducing agents have been used in the transformation of MSCs into cardiomyocytes (11-15). The risk of protein and pathogen contamination in animal-human cell co-culture and the separation of cells other than cardiomyocytes prior to administration are some of the difficulties of this method. Synthetic agents are preferred because they are more stable than protein-based growth agents, have a longer half-life, and do not require living organisms for synthesis. 5-aza has been shown to induce the differentiation of stem cells into a variety of cells, including cardiomyocytes (11, 14, 36). Although the mechanism by which 5-aza promotes cardiomyogenic differentiation remains unclear, it has been shown that it effectively induced the up-regulation of cardiomyogenic specific genes such as cTnT, cTnI, α-cardiac actin and Nkx2 through the ERK pathway (13, 22).

In our study, we used 10 μM of 5-aza to stimulate cardiomyogenic differentiation of hATDSCs. Prior to differentiation experiments, we performed viability assay to test the toxicity of 5-aza on stem cells. Our results suggested that 5-aza had significant toxic effects on the viability of hATDSCs compared to the control group. However, in our four-week differentiation protocol, we observed that 5-aza-treated groups formed a syncytium by making cytoplasmic connections with each other in the three weeks of induction. Furthermore, myotubule-like formations and elongated stick like cells were evident at 4^{th} week. These morphological alterations may be related with the increased expression of proteins supporting cytoskeleton. Furthermore, immunohistochemical analysis clearly revealed that 5-aza could stimulate the expression of cardiomyogenic markers (cTnI, GATA4 and MYH7). In addition, cTnI level was significantly higher in 5-aza-treated groups compared to control group. Our results are in line with studies showing successful differentiation of MSCs into cardiomyocytes by 5-aza treatment (11, 14, 36-38). These studies have confirmed the increased expression of cardiac specific genes such as cardiac troponins, GATA4, myosin heavy chain in 5-aza-treated MSCs. Cardiac troponin I (cTnI) and troponin T (cTnT) are myocardial specific proteins that play essential role in the regulation of cardiac muscle contraction. Transcription factor GATA-4 is a protein which is encoded by the GATA4 gene. It is expressed in the nucleus and functions as a key regulator of mammalian cardiac development. It is known to be highly expressed in cardiac muscle cells throughout the stages of development (39). MYH7 is a gene

encoding a myosin heavy chain beta (MHC-β) isoform mainly in the heart tissue. MHC-β is the main protein including the thick filament in cardiac muscle and plays a major role in cardiac muscle contraction (14).

In the present study, we hypothesized that exenatide, GLP-1 agonist, may have potential effects on cardiomyogenic differentiation and viability of hATDSCs. Our results showed that 5-aza (10 μM) decreased cell viability compared to control, while addition of Exe (10, 100, and 250 nM) to the medium along with 5-aza improved cell viability in a dose-dependent manner. In terms of cytotoxic effects of 5-aza, previous studies in the literature have indicated the relationship between DNA hypomethylation resulting from methyl transferase inhibition with chromosomal instabilities (40) and gene reactivations (41). Also, it was observed that DNA methylation losses also triggered p53-mediated apoptosis (42).

GLP-1 has been investigated in many studies for its cytoprotective and anti-apoptotic effects on different cell types. In these studies, it has been shown that GLP-1 may have an anti-apoptotic effect by inhibiting bax expression in cholangiocyte and neuroblastoma cells (43, 44) and inducing bcl-2 upregulation in PC12 cells and pancreatic beta cells (45, 46). It has also been reported to have a cytoprotective effect by reducing DNA fragmentation in pancreatic beta cells (47). In a more recent study conducted in SHSY5Y neuronal cells, it has been demonstrated that exenatide may have neuroprotective effects against glucose and fructose toxicity (48). On the other hand, several experimental and clinical studies have suggested that GLP-1 and GLP-1 receptor agonists may exert cardioprotective effects against ischemia-reperfusion damage (49-53).

In our study, 5-aza and Exe treated cultures were monitored through 4 weeks for cardiomyogenic differentiation. At the end of the first week, cells in the control group and cultures treated with Exe proliferated and covered the culture dish and had a fibroblast-like appearance. In Exe and 5-aza-treated groups, larger and "rod" or "sphere" shaped cells were observed. On the 3rd week of the cultures, cells in 5-aza-treated groups formed a syncytium by making cytoplasmic connections with each other. On the 4th week, binucleation and multinucleation and myotubule-like formations were also visualized in 5-aza-treated groups. These phenotypic changes in cultures that underwent cardiomyogenic differentiation were consistent with other studies in the literature (38). On the other hand, although some studies (19, 54) have reported spontaneous beating cell populations in the cultures, there are also studies in the literature where this transformation was not observed (55, 56). Therefore, there is no consensus that MSCs can transform into functional cardiomyocytes in vitro in terms of cellular junctions, interaction with other cell types, and electrical activity. Although the reason for these contradictory results in the studies cannot be explained, electrophysiological examination of cells in future studies will provide important findings about the functional development of cells.

Study limitations

There were some limitations of our study. First, we did not measure the mRNA levels of cardiac muscle specific proteins. Second, we did not perform the electrophysiological examination of cells.

CONCLUSION

To the best of our knowledge, this is the first report to demonstrate beneficial effects of exenatide on differentiation of hATDSCs. Findings of our study indicated a combination of 10μM 5-aza with 100 nM Exe was successfully able to induce the expression of cardiac specific proteins that play important regulatory roles in cardiomyogenesis. Also, the treatment of cells with Exe significantly protected cells against the toxic effects of 5-aza by improving the viability and proliferative capacity of hATDSCs. Overall, these findings suggest that co-administration of 5-aza and Exe may increase the probability of regeneration of injured cardiac cells before transplantation by inducing the differentiation potential of stem cells. However, more detailed studies are needed to better understand the mechanisms underlying these effects of exenatide.

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