



Original Article

Positive effect of Periostin on repair of Isoproterenol induced ischemic damaged cardiomyocyte: an in vitro model

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ABSTRACT

Introduction: One of the diseases in developed countries is myocardial infarction that causes the death of many people. A Problem of many new drugs usage concerns their assessment for applying therapeutically for heart disease. Previous studies used animal models but today many researchers tend to apply cellular models for feasibility of cellular model production and application.

Methods: For this purpose, we differentiated human bone marrow mesenchymal stem cells (hBM-MDCs) into cardiomyocyte, then induced damage into cells by Isoproterenol, and finally we assayed repair of cardiomyocyte by Periostin. Damage induction and repair were confirmed by measurement of selected markers for cardiac damage and repair at mRNA level. In addition, we measured LDH activity in culture medium during damage and repair processes.

Results: Our results showed LDH_a and b mRNA levels increased and also cardiac markers decreased during damage of cardiomyocytes significantly. Reciprocally LDH isozymes decreased and cardiac markers increased during repair of cardiomyocytes.

Discussion/conclusion: These alterations in cardiac markers after Periostin treatment demonstrate that Periostin is an effective factor on repair of cardiomyocytes. LDH activity in culture medium decreases after damage induction and increases during repair process. According to our data, Isoproterenol and Periostin are good inducer to produce damaged and repaired differentiated cardiomyocytes respectively.

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1. Introduction

One of the main manifestations of the ischemic heart disease is Myocardial infarction that causes heart failure. Irreversible damage to myocardial cells lead to myocardial ischemia. In spite of heart function and health improvement by clinical therapies, myocardial infarction still remains the leading cause of death worldwide [1].

One of the challenges in investigations about the therapeutic effect of new drugs on injured heart concerns the production of damaged model. Two main models are used for drug treatment studies including animal and cellular models and each have some advantages and disadvantages. Cellular model is the best option for research about cardiovascular diseases and drug treatment to

specially repair cardiomyocytes. Cellular models are preferentially used in cardiac research since the cost of maintenance of cellular model is low, they consume less time, and the drug effect is untranslatable.

Isoproterenol [1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride] is a synthetic material with catecholamine property and β -adrenergic agonist has dual effects at different doses. It can create severe myocardial infarction at high doses and induce heart function improvement at low doses [2,3]. Myocardial necrosis was created after cardiac dysfunction at high dose Isoproterenol via increase peroxidation of myocardial lipids by alterations in cardiac enzymes and antioxidants [4,5]. However, at high dose, Isoproterenol has the ability to deplete energy of cardiomyocyte and alter biochemical and structural functions but in low dose it can act as benefit regulator of heart function. Iso-induced MI was used frequently in many studies for several reasons including extraordinary technical simplicity, excellent reproducibility and low mortality. Iso acts through several channels including energy depletion, lipid peroxidation, and free radical production [3].

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Periostin is known to be an important secreted protein during cardiac development and damage expressed by cardiac fibroblasts [6]. Periostin is known as an important factor which can lead to cardiac development and regulate or indicate pathologies involving the cardiovascular system [7–10]. Periostin is a vital component to induce the maturation and improvement of the tissues of the heart. In addition, Periostin provides the necessary signaling for the in situ creation of the three-dimensional cardiac collagen scaffold during cardiac biogenesis. Periostin is a protein that contains 811 amino acid and is composed of 4 repeating fasciclin domains (Fas1–4), an amino-terminal signal sequence (S.S.) and a putative glycosylation site in the 4th fasciclin domain [11]. Expression of Periostin elevates in the infarct sites of human, mouse, and rat hearts following MI [12,13].

According to recent studies, Periostin treatment may induce endogenous proliferation of cardiomyocyte and enhance the potential for regeneration of mature cardiomyocytes [14]. Periostin contributed to collagen degradation, tissue remodeling, and reduced cardiac fibrosis during the healing process following myocardial damage while preserving tissue matrix [15].

Animal models are often not feasible for studying the events that happen in heart and cardiovascular system. A common approach to study human diseases involves animal models, especially rodents, but due to important biological and physiological differences, this model system may not recapitulate human diseases. Cellular models are used to study heart related metabolic disorders as an alternative approach. The cellular models rely on the use frequently immortalized cell lines or patient-derived fibroblasts. Most applications of iPSC concern differentiation into beating cardiomyocytes to study human heart disease [16,17]. Mice are utilized as a CVDs model for several reasons including easy maintenance in laboratory and the relatively high homology with human genome but their cardio-circulatory system does not perfectly retrace human physiology [18]. Therefore, human cell models are widely used in investigations for their ease of obtainability, unlimited availability, recreating the cellular and extracellular niche of heart and vessels and exhibiting prolonged viability and correct physiology. In previous studies, many cells were used to induce cardiac damage including cardiovascular cells such as primary cardiac mesenchymal cells, C-Kit positive cardiac mesenchymal cells and other cardiac cells.

One of the candidate cells to use as cardiac damage model is cardiomyocyte differentiated from bone marrow derived mesenchymal stem cells. The aim of this study was to improve cellular heart damage model by Isoproterenol and evaluation of its repair. For this reason, first MSCs were differentiated into cardiomyocyte then damaged was induced by Isoproterenol and, finally, Periostin was used to repair the damaged cardiomyocytes.

2. Methods

2.1. hBM-MSC differentiation into cardiomyocyte

In the third passage, 5000 BMSCs were cultured in the each well of a 12-well plate. After 24 h from cell seeding, adherent cells were washed twice with PBS and then fresh DMEM containing 10 μ mol 5-Azacytidine was added. During the differentiation process, a well filled only mesenchymal stem cell without 5-Aza treatment. The cells were incubated in a 5% CO₂ until cardiomyocyte differentiation was performed. During the 3-week period of differentiation process, the plate in the incubator was checked daily and the media was replaced every 3 days. After the differentiation time was passed, validation of the process was determined by cardiac markers expression at mRNA level.

2.2. Induction of damage into hBM-MSCs derived cardiomyocyte

Damage induction on BM-MSCs derived cardiomyocyte was carried out by Isoproterenol. For this purpose, 2.5 mmol from Isoproterenol solution in PBS was prepared and added to each well containing differentiated cardiomyocyte. The plate was incubated for two days. After passing incubation time supernatant was kept to measure LDH level and RNA was extracted for Real Time PCR performance.

2.3. MTT assay

To determine the cytotoxicity effect of Isoproterenol on cells, the MTT assay was done. MTT assay was performed to investigate the viability of cells during the treatment by Isoproterenol on the basis of previous reference. The cells seeded in a 96-well plate and treated with Isoproterenol then 20 μ L of 5 mg/mL MTT solution was added to each well. After incubation of the plate for 4 h, the supernatant was removed and 200 μ L DMSO was added to each well and pipetting was done; finally, the absorbance values were read at 570 nm using a microplate reader.

2.4. Repair of BM-MSCs derived cardiomyocyte Isoproterenol induced ischemia by using Periostin

Periostin was used to repair BM-MSCs derived cardiomyocyte Isoproterenol induced ischemia. To perform this step, Periostin at concentration of 500 mg/mL was added into each well of plate containing differentiation cardiomyocyte then the plate was incubated for 5 days; after this period supernatant was collected and RNA was extracted from cells for conducting Real Time PCR.

2.5. Measurement of expression of cardiac markers and LDH_{a,b} at mRNA level during all steps including cardiac differentiation, damage induction and repair

Cardiac markers mRNA expression level was assayed by Q-PCR. For this purpose, total RNA was extracted from all cell samples by RNA extraction kit in each step. The first strand cDNA was synthesized, then Q-PCR was performed by specific primers as cardiac marker such as Troponin-I, Troponin-T, Connexin43 and α -Cardiac actin in step one based on SYBR Green technique (Table 1). Real Time PCR was done by a mixture prepared from SYBR Green Master Mix, 0.1 mmol of each primer and 100 mg cDNA; then final volume of 20 μ L was achieved by adding deionized water (Table 1 indicated sequence of primers). Program of Q-PCR was primary denaturation at 95 °C for 10 min and followed by conditions: 40 cycles of denaturation at 95 °C for 15 and annealing at 60 °C for 1 min. GAPDH was used as housekeeping gene. In this study all PCR tests were carried out in triplicate and fold changes were calculated on the basis of $2^{-\Delta\Delta Ct}$ formulate.

2.6. LDH activity assay

During the damage and repair of cardiomyocyte after treatment with Isoproterenol and Periostin respectively, we detected LDH activity by Padco Kit according to the manufacture manual.

3. Results

In this study we evaluated the effect of complementary protein Periostin on the repair of Isoproterenol induced ischemic cardiomyocyte. After differentiating hBMSCs into cardiomyocyte, these cells were affected by Isoproterenol in upper therapeutic dose for ischemic damage induction. After examining several doses of

Table 1
Sequence of primers used for real time PCR.

GENE	Forward primer sequence	Reverse primer sequence	Product size (bp)
GAPDH	GACAACAGCCTCAAGATCATCAG	ATGGCATGGACTGTGGTCATGAG	122
LDH-A	GCCCGACGTGCATTCCCGATTCTT	GACGGCTTTCTCCTCTTGCTGACG	361
LDH-B	GGCAACAGTTCCAAACAATA	TAAGAATGTCCACTGGGTTG	386
Connexin43	CCGTACCACAGGCATTGTTT	GACAAAGGAGTAGCCACGCT	138
α -cardiac actin	AAGCAAAAGAGTGGTGCCAG	CCAGCAGCAGGATTCGGAAA	166
Troponin-I	CCAGGGCAGAAGAAGATG	CCACTCTCTCCATCGG	135
Troponin-T	GACACCGAGAAGGAAAACC	GGCAGTAGGCAGGAAGG	114

Isoproterenol and determining their toxicity through MTT test, we selected dose 2.5 mmol to induce the ischemic damage. Cardiomyocyte markers' expression at mRNA level were detected by Real Time PCR to confirm the accuracy of differentiation, damage induction and repair. Undifferentiated stem cells and untreated cardiomyocyte were used as the control group. Cardiac markers used for confirmation of differentiation process included Connexin43, α -Cardiac actin, Troponin-I, Troponin-T and GAPDH as internal control. In addition to cardiac markers, LDH a and b were measured at mRNA level and also its secretion in culture medium was assessed. O-PCR analysis of Cardiac markers expression at mRNA level showed that all markers level increased during differentiation (Fig. 1). Fig. 2 shows alteration of gene expression of cardiac markers at mRNA level during damage and repair. According to Fig. 2, expression of all cardiac markers decreased after

ischemic induction and increased during repair of cardiomyocytes. The mRNA level of LDHa and b elevated in cardiomyocytes after treatment with Isoproterenol but decreased after being affected by Periostin. In addition to mRNA level of LDH, we measured its activity and showed the activity of LDH is reciprocally related to its mRNA level as shown in Fig. 4. As mentioned above MTT assay was performed for the determination of viability of cells Fig. 5 showed result of MTT assay in differentiated, ischemic and repaired cardiomyocytes (see Fig. 3).

4. Discussion

This study describes in vitro induction of damage into cardiomyocytes by Isoproterenol and its repair after treatment with Periostin. This investigation was done during two steps. In the first

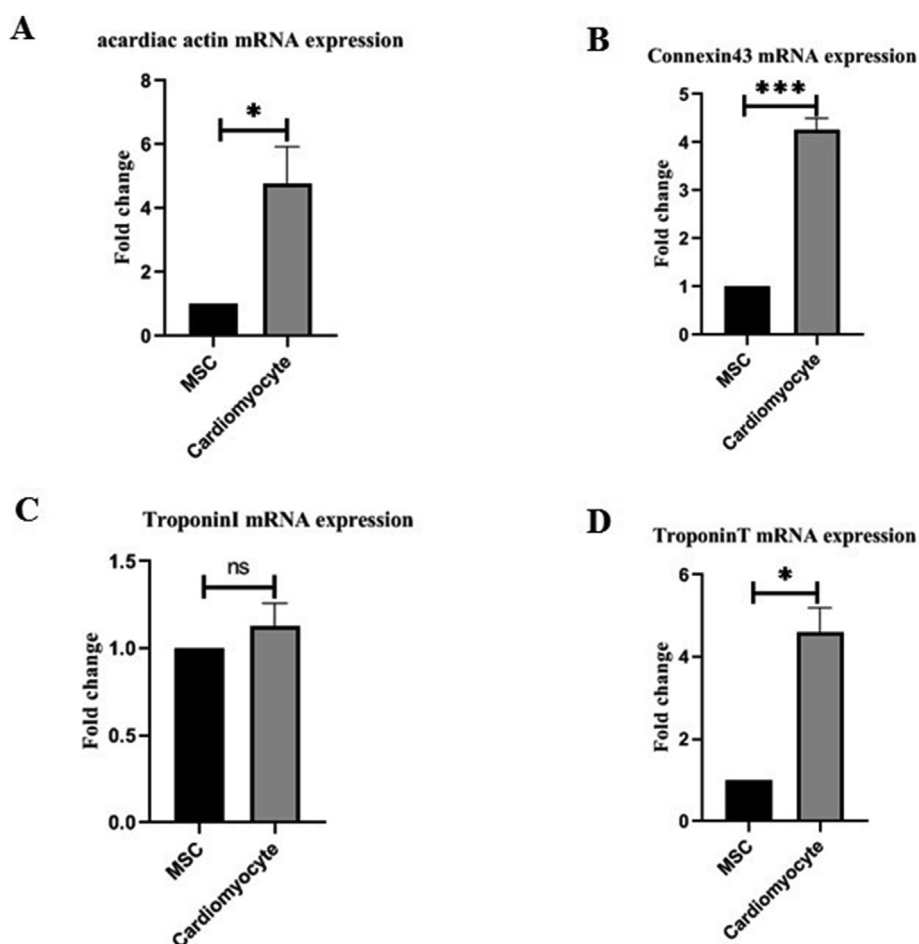


Fig. 1. Expression of cardiac markers at mRNA level during cardiomyocyte differentiation. A) α -cardiac actin B) Connexin43 C) Troponin-I D) Troponin-T. * means P value $0 < 0.05$, *** means P value 0.001, ns means no significance.

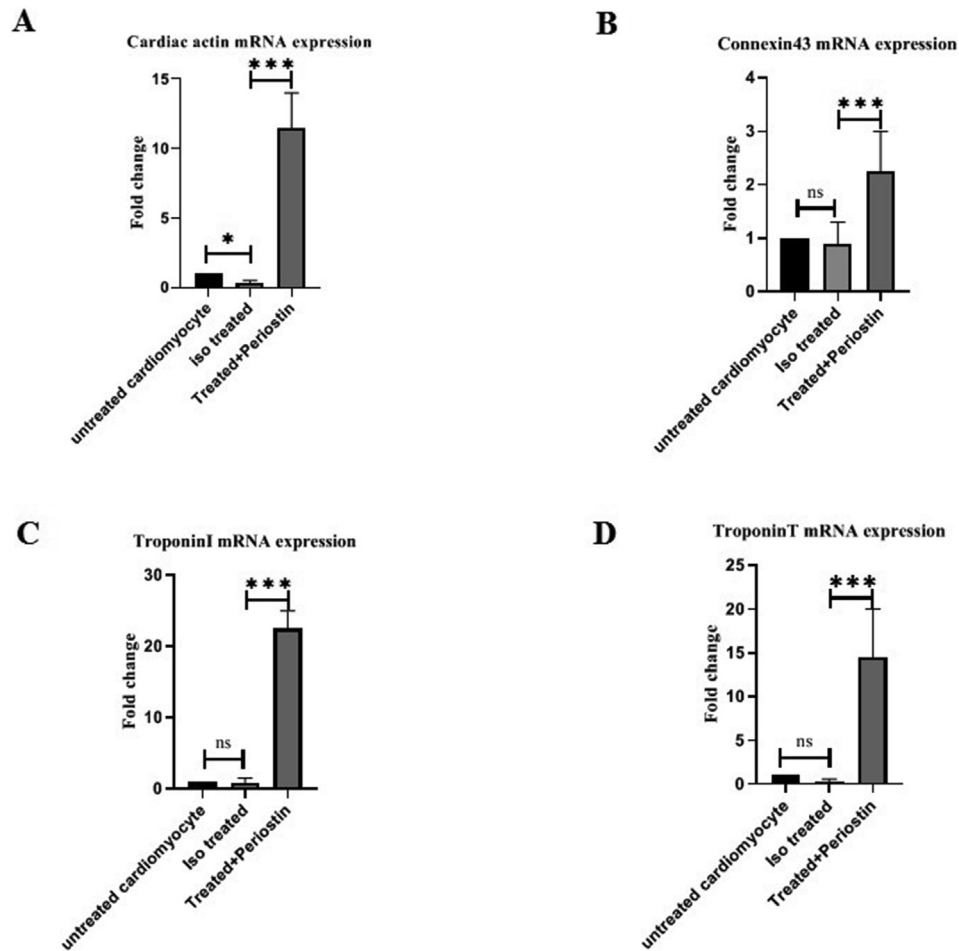


Fig. 2. mRNA expression of cardiac markers during damage and repair of cardiomyocyte. A) α -cardiac actin, B) Connexin43, C) Troponin-I, D) Troponin-T. All of results are presented as Mean \pm SEM, $P < 0.05$. *** means $P < 0.001$, ns means no significance.

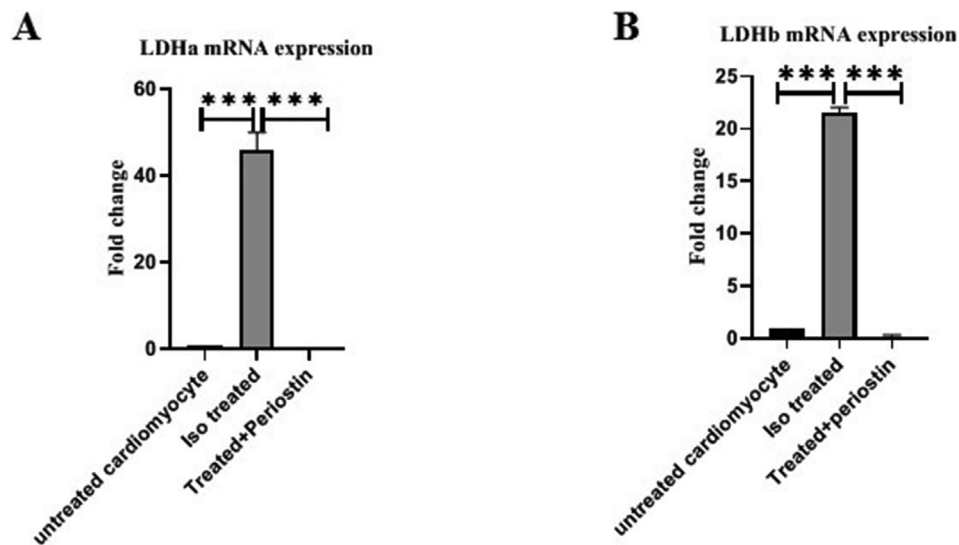


Fig. 3. mRNA expression of cardiac damage markers during damage and repair of cardiomyocyte. A) LDHa, B) LDHb. *** means $P < 0.001$.

step, we performed the cardiomyocyte differentiation from hBM-MSCs and induced ischemic damage into cardiomyocytes by 2.5 mmol Isoproterenol. Then we treated damaged cardiomyocytes

by 500 mg Periostin during the second step. To this end, in first step cardiomyocyte was created from hBM-MSCs in culture medium by using 5azacytine, a cardiogenic factor. In the next step, the obtained

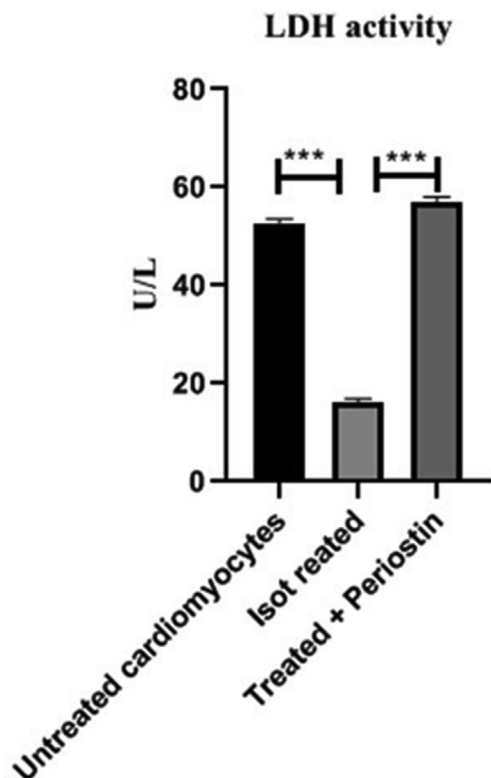


Fig. 4. LDH activity during damage and repair of cardiomyocyte. After treatment of cardiomyocytes with Iso, LDH activity is lower than untreated cardiomyocyte. After treatment by Periostin, LDH activity increased in relation to Iso and untreated cardiomyocytes *** means $P < 0.001$.

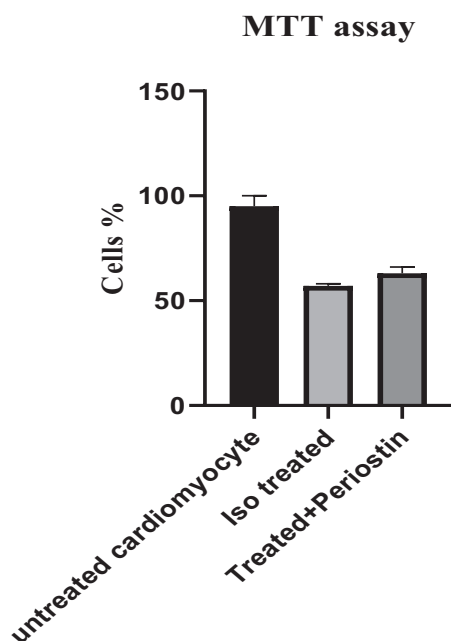


Fig. 5. MTT assay shows about 90% of untreated, 60% Isotreated and 70% periostin treated cardiomyocytes have viability.

cardiomyocytes were affected by Isoproterenol to induce ischemic damage into cardiomyocytes. In the final step, the damaged cardiomyocytes were treated by 500 mg Periostin. Our results showed the cardiomyocyte markers such as α -cardiac actin, connexin43,

Troponin-T and Troponin-I at mRNA level increase after treatment of cells with 2.5 mmol Isoproterenol. In addition to cardiac markers elevation, we demonstrated that LDHa and b at mRNA level increase after induction of damage and decrease after being affected by Periostin but LDH activity changes were reciprocal in related to mRNA level.

Chen et al., 2017 showed Periostin in the infarcted area was upregulated in response to MI in adult and neonatal mice and our finding is in line with studies of Chen et al. [19]. Periostin plays important roles during cardiac development and in the epithelial-mesenchymal transition [20]. It was also linked to cardiovascular diseases such as dilated cardiomyopathy and MI [21,22]. Onur Kanisicak et al. [23] showed myofibroblast, as a periostin-expressing cell type, is necessary for adaptive healing and fibrosis in the heart. In line with previous studies, our results show that periostin has positive effect on repair of cardiomyocyte since all cardiac markers increased after periostin treatment [23].

In addition, our results revealed that Isoproterenol can be used to induce cardiac damage. One of the advantages of myocardial damage induction with Isoproterenol is minor modifications of heart during injury [3].

According to our data LDHa and b expression at mRNA level increased in cardiomyocytes after treatment with Isoproterenol. This finding is completely in line with what is happening in the human body. This shows that Isoproterenol is a good cardiomyocyte damage inducer. The alterations of LDH activity amount was reciprocal with LDH mRNA level. Contrary to data for mRNA level of LDH, activity of LDH decreased unexpectedly after Iso treatment and increased after affected by periostin. This data was opposite to the findings obtained from other studies. The reason of this occurrence during Iso treatment may be attributed to the destruction of LDH mRNA before conversion to protein which we could not detect. Also, during repair process, probably cardiomyocytes quality improved and LDH activity elevated and was higher than in untreated and Iso treated cardiomyocytes. Another important result of this research was setting up cellular model to create damaged cardiomyocyte for cardiovascular diseases and therapies studies. Jimenez and Jimenez 2019 showed cellular model is an important tool to investigate human genetic cardiomyopathies [24]. Because cellular model creation is easy, its proliferation capacity is controllable and its cost of maintenance is low and genetically manipulated cardiomyopathy can be produced easily.

In previous studies, Isoproterenol was applied frequently for several reasons including 1) alterations in heart tissue after treatment with Isoproterenol were similar to those in human body after MI and 2) simplicity of production of this model as well as low mortality. For this reasons, we induced ischemic damage to cardiomyocyte by Isoproterenol. Our data shows Isoproterenol can be a good inducer of cardiac ischemic in cellular model. In addition we demonstrated Periostin has positive effect on repair of damaged cardiomyocyte.

5. Conclusion

This study describes in vitro induction of damage into cardiomyocytes by Isoproterenol and its repair after treatment with Periostin. Our results indicate that Isoproterenol can be a good inducer of cardiac ischemic in cellular model. In addition, we demonstrated Periostin has a positive effect on repair of damaged cardiomyocyte.

Declaration of competing interest

The authors declare there is no conflict of interest.

Acknowledgment

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