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Treatment of Induced Mesenchymal Stem Cells in Ischemic Heart Diseases: Hypoxia

İskemik Kalp Hastalığında İndüklenmiş Mezenşimal Kök Hücre Tedavisi: Hipoksi

Özer Aylin Gürpınar and Irmak Dal*
Department of Biology, Hacettepe University, Ankara, Türkiye.

ABSTRACT

When traditional methods fall short in treating ischemic heart diseases caused by reduced blood flow to the heart due to narrowed coronary arteries, alternative solutions such as cellular therapies are thought. Mesenchymal stem cells (MSCs) are advantageous due to their ease of isolation, migration, and immunomodulatory properties. Preconditioning, which involves regulating cell functions through external stimuli, enhances the effectiveness of cellular therapy. Hypoxia, known as oxygen deprivation, is known to regulate cell survival, migration, and differentiation capabilities. This review explores the current state and future of hypoxia preconditioning in enhancing the therapeutic effects of stem cells.

Key Words
Mesenchymal stem cells, ischemic heart diseases, preconditioning, hypoxia

ÖZ

Koroner arterlerin daralması sonucu kalbe kan akışının azalması ile oluşan iskemik kalp hastalıklarının tedavisinde geleneksel yöntemlerin yetersiz kalması durumunda, hücresel tedaviler gibi alternatif çözümlere başvurulmaktadır. Mezenşimal kök hücreler kolay izolasyon, göç ve immünmodülatör özelliklerinden dolayı avantajlıdır. Hücrelerin dışarıdan bir uyarı ile fonksiyonlarının düzenlenmesi prensibine dayanan ön koşullama, hücresel tedavinin etkinliğini arttırmaktadır. Oksijen azlığı olarak bilinen hipoksinin hücrelerin hayatta kalma, göç ve farklılaşma yeteneklerini düzenlediği bilinmektedir. Bu derlemede, kök hücrelerin terapötik etkisini arttırmakta kullanılan hipoksi ön koşullamasının mevcut durumunu ve geleceğini araştırdık.

Anahtar Kelimeler
Mezenşimal kök hücre, iskemik kalp hastalığı, önkoşullama, hipoksi.

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Correspondence to: I. Dal, Department of Biology, Hacettepe University, Ankara, Türkiye.
E-Mail: irmakdal97@gmail.com

INTRODUCTION

Ischemia refers to the condition where blood flow to a specific region is blocked due to vessel occlusion. Ischemic heart disease (IHD) occurs when narrowed coronary arteries reduce blood flow to the heart muscle [1]. This narrowing can result from atherosclerotic plaque formation or non-plaque-related causes [2,3]. The reduction in blood flow caused by atherosclerotic plaque formation creates an imbalance between myocardial oxygen demand and supply, leading to ischemia. When narrowing is less than 50%, there is no decrease in blood flow; however, if atherosclerosis progresses untreated, symptoms such as chest pain, cold sweats, nausea, and vomiting may occur [2,3]. Risk factors such as smoking, hypertension, diabetes, and inflammation accelerate atherosclerotic plaque development in coronary arteries [4]. Non-atherosclerotic causes include impaired microcirculation or arteriolar irregularities, coronary embolism, reduced blood oxygen content, and conditions that increase myocardial oxygen demand, such as exercise or emotional stress [5]. Ischemic heart disease, characterized by transient pain (angina), arrhythmia, myocardial infarction, and heart failure, is a significant cause of morbidity and mortality worldwide [6,7]. In Europe, IHD causes over 17 million deaths annually, accounting for 20% of all deaths [8].

The treatment for IHD involves managing risk factors like hypertension, smoking, diabetes, alcohol use, obesity, lack of physical activity, and unhealthy diet. Adopting a healthier lifestyle reduces the risk of new cardiovascular events [9]. Treatment options also include medication and revascularization. Revascularization is preffered to reduce symptoms, prevent the progression of atherosclerosis, and prevent atherothrombotic events [5].

Although these treatments are effective in restoring blood flow to the heart and preventing increased damage caused by ischemia, they are limited in their ability to reconstruct damaged tissue after blockage [10]. In patients with multiple vessel disease, surgical coronary revascularization treatments do not provide long-term improvement and often require repeat revascularization. Heart transplantation has recently been effective, but due to the scarcity of donor hearts, patients may have to wait a year or more [11]. Current data suggests that medication that protects the heart at the cellular level is crucial to prevent recurrence and progression of the disease [12].

Consequently, alternative therapies to repair viable tissue in the damaged heart are being explored [13]. Despite the use of various cell types in tissue engineering studies, MSCs are preferred due to their ease of use [14-16]. MSCs are advantageous because they can be easily isolated, have migration ability, and possess immunomodulatory effects [17]. Using these stem cells in cellular therapy can induce the secretion of trophic factors, cytokines, and growth factors that increase cell survival rates in the damaged area. There are many studies on the application of MSCs in treating ischemic heart disease.

One issue in cellular therapy is the apoptosis of applied cells due to their inability to adapt to the damaged area. This decrease in cell number can reduce treatment effectiveness. To prevent this, cells are subjected to preconditioning involving heat shock, oxidative stress, or hypoxia before application. The goal of hypoxic preconditioning is to increase the adaptability and viability of cells to the damaged area. Studies in the literature have shown that hypoxic preconditioning helps MSCs maintain their survival, migration, and differentiation capabilities [18]. This review aims to investigate the current state and future of using hypoxic preconditioning to improve stem cell therapy in ischemic heart disease.

Treatment approaches

Pharmacological approaches

Antithrombotic therapy is considered an effective treatment method for patients with acute coronary syndrome [19]. The drugs used inhibit the formation of thrombin, which suppresses blood clot formation. The goals of this treatment are to prevent the development and progression of thrombosis, support the resolution or stabilization of mural thrombi, and prevent outcomes such as death, myocardial infarction, and stroke [13].

Coronary artery bypass graft surgery

This surgical procedure aims to restore the blood supply and oxygenation of the myocardium, which is deprived of blood due to coronary atherosclerosis, using arterial and venous grafts. Long-term follow-up results of procedures using saphenous veins have reported some disadvantages, including the risk of restenosis over time. In contrast, procedures using arteries have shown higher success rates in both short and long-term follow-ups compared to those using veins [20].

Percutaneous coronary intervention

Since it was first performed by German cardiologist Andreas Grutzig in 1977, it has shown significant advancements in terms of techniques, devices, and medications. Procedures performed during percutaneous coronary intervention (PCI) include brachytherapy (using radioactive sources to prevent restenosis), balloon angioplasty, and stent implantation. This procedure typically reduces the mortality rate of coronary artery diseases compared to treatments involving thrombolytic agents and is generally effective in acute heart attacks [21]. Especially the use of drug-eluting stents has become a frequently used intervention tool, preventing complications like restenosis, which leads to re-narrowing of the artery [13,22]. However, despite reducing restenosis formation, these coronary artery interventions have not shown a decrease in mortality rates associated with ischemic heart disease [12].

Cellular therapy

Cellular therapy generally involves the processes of repairing and regenerating damaged tissues and organs using stem cell treatments [23]. Stem cells are undifferentiated cells with high proliferation capacity. They are classified into four types based on their differentiation capacities. Totipotent stem cells are formed by the fusion of egg and sperm cells and can differentiate into both embryonic and non-embryonic cells (like the placenta). These cells can form an entire organism and have unlimited differentiation capacities. Pluripotent stem cells, derived from inner cell mass of embryo, can differentiate into cells from the three germ layers (ectoderm, endoderm, and mesoderm). Multipotent stem cells, although having limited differentiation capacities, can migrate from their resident tissues and contribute to the healing process of damaged tissues upon stimulation. Finally, unipotent stem cells (progenitor stem cells) can only differentiate into cells of their resident tissues and can repair those tissues to a limited extent in case of damage [24].

In cellular therapies, mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs) are used. ESCs have the ability to differentiate into all cell types; however, their usage is limited due to ethical issues related to their production and risks of immune rejection. iPSCs also possess similar capabilities, but the involvement of viruses in their production processes can lead to unwanted cancer formation or immune rejection. MSCs, on the other hand, are preferred due to their ease of collection, lower tendency for genetic changes, and ability to support tissue regeneration. MSCs can be isolated from various tissues and can migrate to damaged areas. Therefore, MSCs are therapeutically advantageous in the treatment of various diseases [25]. Among all stem cell types, MSCs are one of the frequently used multipotent stem cell types in treating cardiac diseases [26].

Since the first use of stem cells in heart failure treatment in 1998, the number of clinical trials has significantly increased [27]. Recently, cellular therapy has been widely preferred for treating ischemic heart disease to regenerate the damaged part of the heart using stem cells [27]. Stem cells can be easily obtained from different sources and do not require immunosuppressive agents before transplantation. Additionally, they are considered safer, more applicable, and capable of promoting heart repair, making them a focus of interest [26]. Orlic et al. showed that treatment with bone marrow-derived stem cells after acute myocardial infarction improved myocardial function and enhanced heart regeneration nine days after treatment [28]. Following this study, further research has been conducted with other types of stem cells to enhance heart regeneration [29].

Mesenchymal stem cells and their applications

Isolated MSCs can differentiate into multiple phenotypes both in vitro and in vivo [30]. In adults, bone marrow and adipose tissue are the primary tissues where MSCs are abundant. Bone marrow stem cells, due to their versatility and ease of isolation, have been used in treating ischemic heart disease and have shown positive results [14,28]. Hematopoietic cells in the bone marrow also play a role in the process of angiogenesis [31]. Non-hematopoietic cells in the bone marrow, known as MSCs, can differentiate into bone, cartilage, adipose tissue, and connective tissue cells. Besides bone marrow, adipose tissue is another significant source of MSCs, which are advantageous due to their availability, ease of isolation, and low cost. Additionally, their neoangiogenesis activity makes them a preferred choice in treating ischemic heart disease [14,32,33]. MSCs express MHC Class I antigen, giving them immunosuppressive properties, while low-level expression of MHC Class II antigen gives them immunomodulatory properties. By secreting paracrine factors such as cytokines, chemokines, and growth factors, MSCs enhance endogenous cardiac regeneration and contractility, and they also exhibit

anti-apoptotic and anti-fibrotic properties [34]. One of the secreted factors, vascular endothelial growth factor (VEGF), promotes angiogenic differentiation [35]. Therefore, transplantation of stem cells to the infarcted area contributes to the proliferation of cardiomyocytes, protects cells from apoptosis, and induces angiogenesis in the damaged region [34,36-41].

There are numerous preclinical and clinical studies on the use of MSCs in treating ischemic heart disease [42]. The methods of MSC application include intravenous infusion, intramyocardial injection, transendocardial injection, and intracoronary infusion. The chosen method affects the quality of the treatment [43]. Intravenous infusion is a non-invasive and simple carrier system, but it has disadvantages such as limited cellular migration and differentiation and the possibility of microemboli formation. Intramyocardial injection requires fewer cells but has disadvantages like increased arrhythmia formation and a high risk of death due to the need for surgical application. Transendocardial injection, which allows for targeted cell application, carries risks of myocardial perforation and ventricular arrhythmia. Lastly, intracoronary infusion provides homogeneous cell engraftment in infarct borders but cannot be used for occluded arteries and has a high likelihood of microemboli formation during the procedure [43].

MSCs were first used for cardiomyoplasty by Tomita et al. (1999) in a rat model. Five weeks after transplantation, the animals showed expression of previously unexpressed muscle-specific proteins. Additionally, animals treated with cells pretreated with agents known to increase differentiation capacity showed improvement in heart functions [44]. In a study where autologous bone marrow stem cells were injected into patients undergoing transmyocardial revascularization and coronary artery bypass graft surgery, it was found that contractions in the injection sites strengthened compared to the beginning [45]. In a study on the use of MSC therapy in ischemic heart disease, a decrease in mortality rates was observed in patients followed up after cell therapy. The findings indicated that patients receiving stem cell therapy experienced fewer heart attacks and arrhythmias [39,46]. In the POSEIDON study on patients with functional impairment due to ischemic cardiomyopathy, autologous and allogeneic bone marrow MSCs were applied via transendocardial injection. Follow-up showed a reduction in infarct size for both cell types [47]. In the PROMETHEUS study, autologous bone marrow MSCs were administered via intramyocardial injection. Follow-up results showed a reduction in infarct size in patients receiving cell therapy compared to the placebo group [48]. However, the findings were limited due to the small number of participants.

In a recent study, patients with ischemic myocardial infarction were treated with transendocardial injection of autologous bone marrow MSCs and cardiac progenitor cells. The results indicated that the application of both cell types separately and together improved patients' quality of life and reduced risks such as non-fatal stroke and cardiovascular death [49]. In another study, autologous bone marrow MSCs were injected via intramyocardial injection into patients with left ventricular dysfunction after myocardial infarction. The results showed that stem cell therapy improved heart function and reduced infarct size [50].

Two clinical trials on the use of MSCs in ischemic heart disease are ongoing. One study involving 30 participants is using allogeneic MSCs intravenously for diabetes and ischemic heart disease (clinical trial no: NCT04776239). Another study is applying human umbilical cord-derived MSCs intravenously to patients with ischemic heart disease (clinical trial no: NCT04996966).

In a study on pigs with myocardial infarction, it was reported that transendocardial injection in MSC therapy reduced infarct size, while intramyocardial injection, intravenous infusion, and intracoronary infusion showed no improvement. Additionally, researchers stated that the optimal range for transendocardial injection is 20-100x10^6 cells [51].

The number of transplanted cells affects the treatment's effectiveness. In the TRIDENT study on patients with ischemic cardiomyopathy, a dose-dependent comparison of bone marrow MSCs was performed. One group received 20 million cells via transendocardial injection, while the other group received 100 million cells and was followed up. The results showed a reduction in infarct size in both groups, while only the group receiving 100 million cells showed an increase in left ventricular ejection fraction [52]. According to this study, increasing the number of cells applied enhances the treatment's effectiveness. In addition to directly using stem cells, some studies have investigated the effect of applying stem cells in combination with other components or preconditioning to improve the treatment.

Applications of induced mesenchymal stem cells

In a study, it was reported that the application of MSCs overexpressing insulin-like growth factor-1 (IGF-1) increased cell survival by reducing β-catenin expression and protecting cells from apoptosis (Lin et al. 2020). Zhang et al. (2019) reported that the application of MSCs together with the asprosin prevented cell apoptosis in the hypoxic region (Zhang et al. 2019). Another study indicated that the transplantation of bone marrow-derived MSCs combined with bone morphogenetic protein (BMP)-2 and salvianolic acid B (Sal-B) promoted better differentiation of these cells into myocardial cells [53]. In another study, it was stated that the application of rat MSCs modified with Akt to ischemic rat myocardium restored heart performance [26]. He et al. (2019) observed that using adipose tissue MSCs treated with resistin (an adipose tissue-specific secretory factor) in C57BL/6J mice with ischemic myocardium had a positive contribution in reducing myocyte apoptosis [26].

Preconditioning involves exposing cells to heat shock, oxidative stress, or hypoxia before use. Heat shock preconditioning is used to increase cell survival rates. Heat shock treatment has been observed to increase the survival rates of Sca-1(+) stem cells in an ischemic environment, reduce apoptosis in ischemic myocardium treatment, and improve heart functions [18]. In one study, MSCs cultured at 43°C produced more heat shock proteins (HSPs), increasing cell survival rates [54]. Another known cause of apoptosis in injected stem cells is oxidative stress [55]. Therefore, it has been suggested that preconditioning stem cells with H2O2 could increase their therapeutic effects by improving their adaptation to challenging conditions. In a study using MSCs preconditioned with 200μM H_2O_2, it was observed that there was ~25 times more IL-6 secretion in the environment, which could enhance cell migration and proliferation capabilities [18].

In this review, we investigated the effects of hypoxic preconditioning on MSC therapy in ischemic heart disease. The goal of hypoxic preconditioning is to increase the adaptability of the applied cells to the challenging environment of the damaged heart. Hypoxic preconditioned MSCs have been reported to show better attachment, survival, and differentiation capabilities [18]. Researchers have stated that preparing MSCs in hypoxic environments and using them in ischemic heart disease reduces infarct size [29]. In one study, it was reported that the risk of cardiac death after myocardial infarction was lower in mice treated with hypoxic preconditioned MSCs compared to those receiving non-preconditioned stem cell therapy [56]. Yu et al. (2017) suggested that hypoxic preconditioning of MSCs enhances their therapeutic effects on rodents [18].

Applications of hypoxia-induced mesenchymal stem cells in ischemic heart diseases

One of the challenging aspects of stem cell therapy is the low survival rate of cells post-transplantation. This could be due to the challenging microenvironment that cells face due to ischemia, inflammation, oxidative stress, and immune response. In a study on rats with a myocardial infarction model, it was reported that the transplanted stem cells had low viability. This was attributed to the cells' inability to integrate into the tissue [29,57]. If stem cell transplantation used in the treatment of ischemic heart disease can be made more successful, significant advancements could be made in treating cardiac diseases in clinical settings.

To enhance the benefits of stem cell therapy in the damaged heart, applications such as drug therapy, genetic modifications, exosome therapy, and microRNA therapy are used [18]. To prevent the apoptosis of transplanted cells and increase their survival rate, preconditioning the cells before transplantation is recommended. The theory behind hypoxic preconditioning is to expose the stem cell to non-lethal levels of cell stress, ischemia, or hypoxia before transplantation, thereby activating intracellular mechanisms that provide protection against future trauma [18,56].

The lack of optimization in hypoxic preconditioning hinders the understanding of changes that occur in the stem cell post-hypoxia. The duration and frequency of hypoxia exposure affect the metabolic rate and reactive oxygen species (ROS) production in the cell. In one study, it was found that mitochondrial ROS production decreased in myoblasts exposed to two 30-minute anoxia sessions, whereas it increased in myoblasts exposed to anoxia for 1, 2, and 3 hours [29]. The optimal duration of hypoxic preconditioning varies depending on the cell type and procedure. In one study, differences in apoptosis degrees of endothelial progenitor cells exposed to hypoxic conditions were observed at 48 and 72 hours, while no differences were seen at 24 hours. Another study reported that even short periods of hypoxia exposure, such as 6 hours, could accelerate apoptosis in some cell lines [29]. In one study, the optimal duration and oxygen concentration of hypoxia were

determined. Cells were exposed to hypoxia with 2% and 5% oxygen concentration for 10 days and 48 hours. The results showed that short-term hypoxia exposure increased the anti-inflammatory effects of MSCs, enhancing their therapeutic effects [58]. Kim et al. (2011) reported that MSCs exposed to hypoxia for 2 weeks showed more proliferation and survival behavior compared to normoxia [58]. A study has determined that cell survival and DNA repair are higher in MSCs exposed to 2%-5% oxygen concentration [59]. In a study involving MSC-derived EVs, it was stated that EVs obtained from MSCs exposed to 1% oxygen concentration for 3 and 6 hours were more effective in wound healing compared to EVs obtained under normoxic conditions [60]. Chacko et al. (2010) has found that the expression of the cell survival protein survivin and the cell cycle regulatory protein p21 decreased after 72 hours of hypoxia exposure but was upregulated after 24 hours of exposure. MSCs exposed to 0.5% oxygen concentration for 24 hours showed a high level of apoptosis, while those exposed to 0.1% oxygen showed a lower rate of apoptosis. The lowest rate of apoptosis was observed in cells grown in a normoxic environment [61]. MSCs grown under 0.5% oxygen concentration were found to provide a significant increase in angiogenesis and cardiac function after transplantation compared to cells grown under normoxic conditions [61]. Researchers have determined that MSCs grown under 0.5% oxygen concentration provide a significant increase in angiogenesis and cardiac function after transplantation compared to cells grown under normoxic conditions [62].

Although the differences in degrees of hypoxic preconditioning have not been fully discovered, the changes observed in MSCs after preconditioning in the literature can be summarized as follows:

Effects on cell viability and apoptosis

Hypoxic preconditioning increases cell viability rates as well as the expression of anti-apoptotic genes. In stem cells exposed to hypoxic conditions for 6 hours, the expression of Bcl-2, an anti-apoptotic factor, increased while active caspase 3 decreased [29]. Similarly, in a study where stem cells were cultured in hypoxia for 48 hours, MTT analysis results showed increased cell viability levels, while levels of caspase and lactate dehydrogenase, indicators of cell death, decreased [63]. Kubo et al. (2008) reported low levels of ROS in cells preconditioned with hypoxia using the cell metabolism inhibitor LY-83583 [29]. In one study, it was stated that mitochondrial membrane potential, which helps determine apoptosis, was preserved due to reduced cytochrome c release following hypoxia [29]. The obtained results suggest that hypoxic preconditioning can help MSCs cope with stress in ischemic heart tissue.

Effects on protein expression

There are studies investigating the effects of hypoxia exposure on protein expression in cells. p38 proteins play roles in cell growth, viability, proliferation, and apoptosis. p38 can increase the expression of calreticulin, a chaperone protein involved in the cell's response to stress conditions. Considering the increased phosphorylation of this protein in cardiomyocytes exposed to ischemia, this protein family may play an important role in protecting the heart under hypoxic conditions [29]. Many secreted proteins such as endothelial nitric oxide synthase (eNOS), fibroblast growth factor-2 (FGF-2), hepatocyte growth factor (HGF), and insulin-like growth factor-1 (IGF-1) are expressed at high levels under hypoxic conditions [29]. It has been determined that the proangiogenic growth factor VEGF showed a significant increase after 48 hours of hypoxic preconditioning. Researchers have also determined that the expression of CXCR4, a receptor for factors related to cell migration such as SDF-1 and HGF, angiotensin, increases with hypoxic preconditioning [61,64]. Inhibiting the intracellular pathways involving these proteins can provide insights into their roles in heart repair or the mechanisms of heart repair.

Effects on angiogenesis

Preconditioned stem cells not only show higher survival rates but also produce more angiogenic factors [29,65-68]. Additionally, hypoxic stress plays a role in regulating genes and proteins related to the differentiation of MSCs into cardiomyocytes [29,69]. By stimulating hypoxia-inducible factor-1α (HIF-1α), a transcriptional regulator important in pathways like angiogenesis and heart protection, preconditioning can protect myocytes from reperfusion injury [29,56,70]. VEGF, regulated by HIF-1α, plays a significant role in angiogenesis resulting from ischemia and hypoxia and in repairing damaged tissue [70]. VEGF expression increases in cardiac myocytes and arteriolar smooth muscle cells after myocardial infarction. In vivo studies have shown that hypoxia increases the expression of cardiac HIF-1α and VEGF [56,65,71]. Therefore, hypoxic preconditioned stem cells are expected to improve heart function permanently and reduce infarct size [29,72]. Infarct size is a useful parame-

ter for determining the severity of damage caused by myocardial infarction. In one study, it was determined that infarct size decreased in treatment with bone marrow stem cells exposed to anoxic preconditioning [29]. Although this process is not fully understood, it is believed that paracrine signaling plays a role through survival and angiogenic factors secreted by the surrounding damaged tissues. These factors are thought to exert effects through protective mechanisms, stimulation of angiogenesis, and regulation of metabolism. Preconditioning stem cells with hypoxia before transplantation increases the secretion of these factors [29]. In one study, mice treated with hypoxic preconditioned MSCs showed a reduction in QRS duration and resistance to arrhythmia. Researchers suggested that the reduction in mortality rate in ischemic heart disease was related to the decrease in arrhythmia formation after myocardial infarction [56]. Jaussaud et al. (2013) reported that pigs treated with MSCs prepared under hypoxic conditions showed increased capillary density and left ventricular function 30 days after treatment [73]. Studies in the literature indicate that hypoxia stimulates paracrine factors in stem cells, promoting angiogenesis. Researchers observed increased levels of HIF-1α, angiopoietin, VEGF, VEGF receptor, EPO, and EPO receptor after 24 hours of hypoxic conditions in in vivo studies. The increase in these factors accelerated vessel formation in rat heart sections [62,68]. The results suggest that hypoxic preconditioning may support MSC-induced angiogenesis in the damaged area.

DISCUSSION

Ischemic heart disease, which causes dysfunction and death of cardiomyocytes, remains a leading cause of death worldwide despite advances in drug and surgical treatment options [74]. Heart transplantation has recently become the standard treatment for heart diseases; however, its application is limited due to the scarcity of donor hearts and the necessity of lifelong immunosuppression. Surgical interventions are not preferred due to their high cost and potential complications such as bleeding and infection. Traditional treatments like angioplasty and thrombolytic agents can only eliminate the cause of infarction but cannot effectively restore the damaged heart. However, the recently accepted cell therapy can directly regenerate heart tissue by inducing neovascularization and cardiogenesis [75].

Cell therapy involves transplanting human or animal cells to repair damaged tissues or cells. This method can be used to enhance cardiac performance through the differentiation of applied cells into cardiomyocytes. It has been shown that applied cells can induce vascularization through their fusion into new capillaries and perivascular cells. Therefore, it is considered a suitable therapeutic option to activate regenerative mechanisms in ischemic heart disease and heart failure [13,74].

MSCs are considered suitable candidates for treating ischemic heart disease due to their paracrine effects, immunoregulatory properties, and ability to differentiate into heart cells [36,74]. However, during stem cell applications, cell migration to the damaged area, tissue integration, and contributions to the healing process can lead to inefficient results due to cell loss and low retention rates [62,74]. Cell viability rates decrease within 4 days post-application [62]. Therefore, improving the survival of applied cells plays an important role in enhancing the effectiveness and efficiency of stem cell therapy. Recently, many methods, including genetic modifications, suitable transplantation tools, and preconditioning, have been developed to prevent stem cell apoptosis and enhance their therapeutic potential.

Preconditioning is a method applied to activate survival mechanisms of cells with non-lethal stimuli, enabling them to adapt to challenging conditions. Many studies have shown that both pharmacological and mechanical preconditioning increases the survival rate of MSCs after application to the infarcted heart [63]. Severe hypoxia can cause death in certain cell types such as endothelial cells, but short-term hypoxia exposure has been observed to provide cytoprotective benefits. These benefits include increased cell viability and support for angiogenesis, which are critical for cell therapy [63]. Non-lethal hypoxic preconditioning stimulates many endogenous mechanisms, including the expression of proteins that provide protection against future lethal hypoxia and other threats [62]. Additionally, it has been reported that this preconditioning has significant benefits in improving heart function and reducing infarct size [29]. Hypoxic preconditioning suppresses apoptosis through upregulation of Bcl-2 and VEGF and induction of ERK/Akt phosphorylation [63].

In a study using bone marrow-derived MSCs, hypoxic preconditioning was observed to enhance autocrine and paracrine signaling in cells, reducing apoptosis of transplanted cells and endogenous cardiomyocytes [62]. Researchers suggested that the upregulated HIF-1α after hypoxic preconditioning could induce the differentiation of stem cells into cardiomyocytes [76]. Although HIF-1α is beneficial in various situations, its upregulation may inhibit the differentiation of stem cells into live myocardium. In one study, it was observed that hypoxic preconditioning of mouse-derived pluripotent stem cells inhibited their differentiation into cardiomyocytes [77].

Many researchers have reported that hypoxic preconditioning increases in vitro cell proliferation and provides better-preserved stem cell properties [58,78,79]. However, in one study comparing hypoxic preconditioned bone marrow MSCs with non-preconditioned stem cells, no differences were found in terms of cell proliferation and viability [66]. Similarly, although increases in anti-apoptotic proteins like XIAP were observed in one study, no changes were reported in Bcl-2 protein levels after hypoxic preconditioning [80].

In one study, autologous transplantation of bone marrow-derived MSCs preconditioned with different differentiation factors and hypoxia was performed in a rabbit myocardial infarction model. The results showed no significant differences in the therapeutic abilities of differently preconditioned cells. Although no significant differences were found, hypoxic induction of cells is preferred due to the lack of direct chemical processing and cost-effective preparation protocols [36]. In contrast to these findings, some studies in the literature have shown that an oxygen concentration of 1% or less inhibits stem cell proliferation [66,81-83].

In conclusion, non-lethal hypoxia stimulates adaptive responses that support the survival of MSCs. Enhancing the adaptability, survival, migration, and differentiation abilities of stem cells before injecting them into the damaged heart with challenging conditions can improve the success of the treatment. However, more studies are needed to determine the long-term effects of hypoxic preconditioning in the damaged heart and whether it can become a standard application.

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