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Review

Mitochondrial Transplantation and Transfer, from past to future expectations

Mitokondri Nakli ve Transferi, geçmişten gelecek beklentilere

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Abstract

Mitochondria, with their unique roles in cell energy metabolism, continue to be studied by years of research. Mitochondrial transfer can be summarized as the process of transferring isolated mitochondria to the damaged tissue. In this way, it is aimed to improve the mitochondrial dysfunction in areas with impaired mitochondrial functions such as heart damaged tissue. Although there are many studies on this subject, especially cardiomyocytes, the protective effects of the application in processes such as myocardial ischemia and reperfusion injury continues to be investigated. Although there are different procedures for transferring the isolated mitochondrin to the damaged tissue, many studies have reported positive results regarding the application. In this review, it is aimed to look at the subject from a wide window, while examining the studies done in this field in the literature.

Keywords: Mitochondrial transfer, Mitochondrial transplantation, Mitochondria, Tissue injury

Öz

Hücre enerji eldesinde üstlendikleri eşsiz rolleri ile mitokondri yıllardır devam eden araştırmalar ile incelenmeye devam etmektedir. Mitokondriyal transfer hasarlanmış dokuya izole edilmiş olan mitokondrinin transfer edilme süreci olarak özetlenebilir. Bu sayede kalp hasarı olan doku gibi mitokondrial fonksiyonları bozulmuş bölgede mitokondrial disfonksiyon durmunun düzeltilmesi amaçlanmaktadır. Bu konu ile ilgili özellikle kalp dokusunda birçok çalışma olmakla beraber uygulanın miyokardial iskemi, reperfüzyon hasarı gibi süreçlerde koruyucu etkinliği araştırılmaya devam etmektedir. İzole edilen mitokondrin hasarlı dokuya aktarılmasında farklı prosedürler bulunmakla birlikte, birçok çalışmada uygulama ile ilgili olumlu sonuçlar bildirlmektedir. Derlemede literatürde bu alanda yapılmış olan çalışmalar incelenmekle birlikte konuya geniş bir penceren bakılması hedeflenmiştir.

Anahtar kelimeler: Mitokondriyal transfer, Mitokondriyal transplantasyon, Mitokondri, Doku hasarı

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Introduction

Mitochondria are unique structures that can be called energy units for cells, in addition to meeting the energy needed by the body through mitochondrial electron transport chains and oxidative phosphorylation. It can be said that the organelle plays a central role between pathological and physiological issues in terms of associating processes such as homeostasis and energy metabolism in normal tissue with issues such as neurodegeneration and immunity (1).

In general terms; Mitochondrial transplantation can be defined as the transfer of isolated mitochondria to damaged tissue. Mitochondrial transplantation has entered the literature with various experimental studies as a method for the treatment of mitochondrial damage in different tissues and organs. Mitochondrial damage and dysfunction is defined as an important cause of cardiac dysfunction in patients with myocardial ischemia-reperfusion injury. Although this functional change occurs during ischemia, an increase is also observed during restoration of myocardial blood flow and oxygen distribution to the tissue. It is known that this condition shortens the contractions and lifetimes of myocardium (1). The first studies on the method date back to 2009. The results regarding the ischemic heart tissue in the rabbit model were shared in those years. In the light of the data obtained, it is known that isolated mitochondria are transplanted to the damaged myocard in the rabbit model following Langerdorff perfusion (2). With ongoing studies, the method has been defined as mitochondrial transplantation and it has been shared that this application provides a decrease in the levels of biochemical markers observed after myocardial damage in the infarct area, as well as the decrease that can be seen in the basic heart function. In addition to these data, the experiences obtained also indicate that the frozen and thawed mitchondria suffer a loss of function. With previous experiences it is shown that, unlike the studies that will continue, the transferred mitochondria remained in the intercellular space and did not interact with the cardiomyocyte. Although the mechanism of action of mitochondrial transfusion is still not fully explained; It is suggested that the method reduces the levels of oxidative damage measurable by lipid peroxidation products in the lesion area. It is not yet clear whether this reduction is the primary result of the method or a protective effect originating from the mitochondria.

In the next step in experimental animal studies; in vivo effects of mitochondrial transplantation in a rabbit heart ischemia-reperfusion model is experienced. In this study, mitochondrias obtained from skeletal muscle tissue of the same subject were transplanted, and this method led to a decrease in myocardial infarct area in damaged myocardial tissue after 30 minutes of local ischemia, within 2 hours of reperfusion and 4 weeks of follow-up. Regular follow-ups with echocardiography have demonstrated that the heart exhibits normal contractile function 10 minutes after the start of reperfusion in mitochondria transplanted subjects. On the other hand, in the control group, hypokinesia was observed in the ischemic area for 4 hours. With the results of this study also penetration of Mitotraker Red (CMXRos) (a specific staining method for mitochondria) marked mitochondrias in cardiomyocytes and other cells, were detected. With these datas, it was declared that the transplanted mitochondria did not only stay in the intercellular space, but also penetrated into the cells, and it was continued to be investigated in the ongoing studies how the uptake pathways and cell integrity could continue (3).

There are varied data on the in-vitro results of the methods in different experimental models with mitochondria transplantation. For instance, Masuzawa et al. investigated the penetration of mitochondria isolated from human tissue into 2-day-old rat cardiomyocytes. Mitochondria were detected in heart muscle cells in studies with Transmission Electron Microscope (TEM). In the same study, when rat liver tissue was incubated with mitochondria transplanted heart muscle cells for 4 hours; a 2-fold increase in liver cell respiration rate was detected and shared. These results suggested that mitochondria transplantation restores impaired cell energy resources as a result of ischemia/reperfusion (3). In a different study conducted in 2014, researchers transplanted mitochondria into rat heart muscle cells called P0 that did not contain mitochondria, and as a result, they found an increase in respiratory rate and life span in these cells (4). Following this results, in a different model H9c2 cell culture was cultured with mitochondria obtained from the same cell line or from a different cell line, L6 cells. In this study, standard cell culture medium (DMEM) containing more than 1mM Ca+2 was preferred and the medium was supplemented with pyruvate and glutamine. As a result of the experiment, an increase in basal and maximum cell respiration rates was shared in the presence of carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP) (5).

Encouraging results of in-vitro studies have facilitated different experimental models of mitochondrial transplantation for

cardiac ischemia. Results obtained with these studies have declared the protective effect of mitochondria in ischemia model (6) even in the circulation via the coronary artery (7).

In the first studies on mitochondrial transplantation, promising data draws attention. In the light of these data, it is seen that the studies trying to clear up the pathways showing the effect of the procedure were examined in the following years. In 2017 we can see data sharing that transplanted mitochondria; equalizes the functions of endogenous mitochondria with reduced function. This method contradicts the classical information that high calcium levels, which are characteristic of the extracellular media environment, cause loss of mitochondrial function (8). Possible hypotheses that attempt to explain this divergent finding in the following years are explored below.

Promising data on experimental models led to clinical trials. In 2017, important data about the method were shared in the literature. Pediatric cardiac surgeon Sitaram M. Emani and researcher McCully processed mitochondrial transplantation in infants with ischemic heart damage. It was performed in individuals aged between 2 days and 2 years, followed by extracorporeal membrane oxygenation (ECMO). As a result of the application to 5 patients, 3 of them had tolerated the ECMO application for 30-50 hours and were followed up for a few months. After mitochondrial transplantation, which was applied to two 6-day and 4-year-old patients, a significant improvement was observed in the cardiac functions of those patients, but the patients died eventually (9). These data were criticized with cautious optimism in the literature.

In order to better interpret the experimental studies and results related to the method, it is seen that they are classified under 3 headings (1, 10):

1) Pre-Ischemia

2) Before reperfusion

3) During reperfusion

1) Mitochondrial transplantation before ischemia:

The first study with this technique was performed by Guariento et al. Researchers declared the results of female Yorkshire pig model, which the left coronary artery was cannulated and 1 x 109 mitochondria were transplanted once and in every 5 minutes for a total of 60 minutes. 15 minutes after the end of the injection, regional ischemia was occured by clamping the left anterior descending branch for 30 minutes. Subsequently, the protective efficacy of mitochondrial transplantation before ischemia was evaluated by providing reperfusion for 120 minutes. Coronary blood flow and heart ejection fraction values increased significantly 30 minutes after ischemia, and these values remained constant until the end of reperfusion. Significant reduction in infarct area was detected with improvement in heart muscle functions in single and continuous applications. Also, a significant decrease was detected in infarct size/area at risk (IS/AAR) values. No difference was detected when the continuous application was compared with the one-time application. The data obtained were interpreted as mitochondrial transplantation before ischemia can prevent myocardial ischemia-reperfusion injury and reduce mortality-morbidity rates in individuals with ischemia-reperfusion injury (11).

2) Mitochondrial transplantation before reperfusion:

The first experimental model in the literatüre analyzing mitochondrial transplantation before reperfusion was performed in the rabbit model by Mc Cully et al. The research team transplanted the mitochondria they isolated from the left ventricle to the damaged area in ischemic heart tissue. Mitochondria transplantation was completed before Langendorff perfusion in the experiment. With the data obtained by this research results of the technique were shared as cardioprotectivity and improved ATP levels (2). In the following years, the same method was performed in animal in-vivo ischemia-reperfusion injury model by Masuzawa et al. Mitochondrias obtained from autologous pectoralis major were transplanted into the ischemic area formed in the heart tissue. As a result of the experiment, significant reductions in myocardial infarct markers, including creatine kinase MB (CK-MB), cardiac troponin I (cTnI), and caspase-3, were observed in the mitochondria transplant group. Also, a decrease in IS/AAR ratio values was detected in the same study (3). Kaza et al., the same mitochondrial transplantation method was preferred. In the pig model, autologous transplantation of mitochondria obtained from the pectoralis major muscle was performed. Before reperfusion, transplantation was performed to the ischemic remainder formed in the heart, and the results were evaluated with both light and electron microscopy. As a results of this research, it was observed that the damage was not evident in the transplanted group. In addition, while a decrease in myocardial infarction area and the IS/AAR ratio values were reported, there was an increase in CK-MB and cTnI levels in the control group (6).

3) Mitochondrial transplantation during reperfusion:

Blitzer et al. introduced a different cardiac ischemia model in an animal model to investigate mitochondrial transplantation during reperfusion. After 120 minutes of perfusion, autologous mitochondria transplantation was completed. Following the completion of the transplantation, another 120 minutes of perfusion was applied. Ejection fraction, short axis shortening rate and area change score values were found increased with the echocardiographic examination. Also, mitochondrial transplantation application provided during perfusion may cause an increment in long-term morbidity and mortality of patients scheculed for cardiac surgery (10).

Different delivery methods of mitochondria in experimental studies:

1)Using Injection: It can be defined as a simple method applied using Tuberculin syringe with a standard 18-, 28-, or 32-gauge needle or an insulin syringe with a 28-gauge needle (3, 12). This technique is preferable in conditions which there is no need for an additional suture during heart surgeries and it does not cause additional damage to the myocardium. In studies on rabbit heart tissue, mitochondrial structures marked with MitoTracker were detected in the area of injection and 2-3 mm around of it. Transplanted mitochondria were seen in the area close to cardiomyocytes. 1-2 hours after the injection, the mitochondrial structure was found to be integrated into the cardiomyocyte and close to the sarcomere (between the z-line and the sarcomere) . Immune reaction and arrhythmia were not observed contrary to the increase in ATP levels (6, 13). In a different study performed with injection, by Orfany et al., the mitochondrial structure was taken into the cell by actin-dependent endocytosis. Although the injection route is feasible, it should be taken into account that there may be a need for injection into different areas (13, 14) which could be associated with additional damage for cardiac musle tissue.

2)Using Intracoronary Perfusion: In this method, exogenous mitochondria are distributed in a general way via the coronary artery instead of a prominent area. With this technique mitochondria transplantation can be completed for a general area in a short time through the coronary artery unlike the injection method. However, compared to direct injection method, both methods had cardioprotective effects with no significant differences. Considering a wider effect, it can be thought that this method may be a more appropriate choice for cases with a diagnosis of multiple cardiovascular diseases (2, 7, 12, 13, 15).

3) Inherent Mitochondrial Transfer Mechanism in Cells: It can be thought that transfer of mitochondria via stem cell route may be a preferable method for mitochondria-dependent diseases. Bone marrow dependent stem cells are highly effective for this method with their high expression levels. Although Miro1 is a calcium-dependent protein structure, it binds the mitochondrial structure to the dynein protein, allowing the mitochondria to move with the microtubules in the cell. Miro1 expression in stem cells can expand the distribution of donor cells. The view that stem cells can heal recipient cells by mitochondrial transfer can be identified among the different features of the method. In the literatüre it is declared that stem cells with Miro1 expression have wide mitochondrial distribution ability as well as a healing effect on epithelial damage, while this feature has been found to be absent in Miro1 gene knockout (MSCmiroLo) subjects (16, 17). It is known that bone marrow-derived stem cells can be effective in mitochondria transfer in neurons damaged as a result of spinal cord injury. It is stated in the literature that the main mechanism during this transfer is related to the gap junction structure. As a result of transferring mitochondria to neurons with stem cells, there is an increment of ATP levels in the cell and lactate dehydrogenase activation but with a decrease in apoptosis levels. Application of mitochondria transplantation using stem cells may be promising for patients with spinal cord injury (18).

4) Effect of Drug Delivery System: Application methods for mitochondria transfer may have uncertainities for the patient due to their invasiveness. In order to maximize the results of mitochondria transplantation, it is of great importance to develop the use of auxiliary drugs in the transport of mitochondria to the target tissues or organs. Using PEP-1 modified mitochondria structures (PEP-1-MITO) increases the uptake of mitochondria into the target cell. It has been reported that mitochondrial function improved within days, reactive oxygen species (ROS) decreased, and membrane potential improved in cocultures of fibroblast cells obtained from patients with myoclonic epilepsy, provided with PEP-1-MITO, compared to the untreated control group (15, 19). In a different study conducted in the rat Parkinson model, after autologous/allogeneic PEP-1-MITO injection, the exercise capacity of the subjects improved, as well as an improvement in the expression of substantia nigra respiratory chain complex protein (20). In addition to these effects mentioned, the anti-tumoral effect after mitochondrial transplantation supported by PEP-1 is among the data shared in the literature. These effects can be exemplified as inhibition of breast cancer cell proliferation and improvement of chemotherapy sensitivity (21). (Diseases related with Mitochondrial Transplantation are summarized in Table -1 (1))

Table 1: Application of Mitochondrial Transplantation for Various Diseases with related sources of m itochondriai transfer models, results and references (1)

Disease Models	Sources of Mitochondria	Transfer Modes	Results	References
PD	PC12 cells and human osteosarcoma cells	PEP-1-MITO	Mitochondrial complex I protein and mitochondrial dynamics restored	(20)
Type 2 diabetes	Pectoralis major muscles	Infusion through aorta	ATP content increased significantly and myocardial infarction area apparently decreased	(22)
HIRI	Allogeneic liver	Intrasplenic injection	ALT, apoptosis markers, and ROS production decreased	(23)
HL	Hepatoma cells	Caudal vein injection	Serum transaminase activity and cholesterol levels decreased	(24)
ALI	Gastrocnemius muscle BMSCs	Pulmonary artery infusion, tracheal atomization, GJIC	Dynamic compliance and inspiratory capacity significantly increased; alveolar ATP concentration rose	(25, 26)
PAH	Femoral artery smooth muscle cells	Intravenously injected	Inhibited pulmonary vasoconstriction; reduced pulmonary vascular remodeling	(25, 27)
SCI	Soleus muscles	Direct injection in medial gray matter	Acute bioenergetics of maintaining injured spinal cord	(28)
MCAO	hUC-MSCs	ICV	Reduced astrocyte proliferation and microglia activation; reduced infarct size	(29)
Eye disease	MSCs	Cocultivate	Reduced the loss of retinal ganglion cells	(30, 31)
ALI	Muscles of mice	Injected into the muscles of the hind limbs	Improved skeletal muscle injury and 10 enhanced hind limb function	(14)
DD	Hippocampus	Intravenously injected	Significantly reduced the activation 39 of astrocytes, microglia, and neuroinflammatory factors; increased the expression of brain-derived neu- rotrophic factor	(32)
AKI	BMSCs	Injected into renal cortex	Cellular oxidative stress decreased; promoted the regeneration of renal tubular cells	(33)
Mammary cancer	143B osteosarcoma cybrids	PEP-1-MITO	Weakened the vitality of MCF-7 breast cancer cells; improved the sensitivity of chemotherapy	(21)
Infertility	Oocyte precursor cell	Injection into follicular plasma	Increased in vitro fertilization rate	(34, 35)
Septicemia	L6 muscle cells and UC-MSCs	Intravenously injected	Improved the survival rate of spleen in sepsis and bacterial clearance; reduced apoptosis and inflammatory response	(36)

(ALT, alanine aminotransferase; AKI, acute kidney injury; ALI, acute limb ischemia; BMSCs, bone marrow stem cell, DD, dysthymic disorder; HL, hepatic lipidosis; HIRI, hepatic ischemia–reperfusion injury; hUC, human umbilical cord; MCAO, Middle Cerebral Artery Occlusion; MSCs, Mesenchymal stem cells; PAH, pulmonary arterial hypertension; PD, Parkinson's disease; ROS, reactive oxygen species; SCI, spinal cord injury.)

Regarding the mentioned topic, multifunctional mitochondrial targeting liposome nanodevice, ie, the MITO-Porter system was developed by Yamada et al. Considering that the transplanted structure combines with the cell, divides within the cell and shares some biological molecules belonging to the cell, Yamada and his team developed the MITO-Porter and aimed to combine with the mitochondria and be transplanted. The basic mechanism of action can be classified under 3 headings (1):

1) MITO-Porter surface is modified by positively charged cell-penetrating peptide R8.

2) It binds to mitochondria with negative membrane potential via MITO-Porter electrostatic interaction.

3) MITO-Porter associates with the mitochondrial membrane and is transported as a complete structure

All data support that the application of mitochondrial transplantation before ischemia or after reperfusion may be an adjunct method. It is known that the method is effective for ischemic damage in skeletal muscle and lung tissue as well as cardiac muscle (10, 11, 14, 25). In addition, data on the use of the method in the diagnosis of pulmonary hypertension (27, 37), different neurodegenerative diseases (20) and even depression (32) and schizophrenia (38). In addition to the long history declaring the results of the technique and scientific data obtained in different models, the therapeutic

mechanisms related to the mitochondrial transplantation method are still unclear (8). It can be said that the need for studies to explain the effects of the method continues in order for the results of the method to be more predictable and thus to accelerate clinical applications.

Uncertainties about the method

With the help of trial data especially after 2018, it is clear that opinions about the mechanism of mitochondrial transplantation perspective have widened (19). In the light of the hypotheses shared in the literature, mitochondria can remain healthy in the extracellular environment and they can penetrate into cells to repair the impaired ATP production. In the method in which mitochondria are applied with the blood circulation, the mitochondria penetrate the endothelium of the blood vessel before entering the cell (8)

There are basically two questions about the method mentioned by Mc Cully et al. The first of these is the continuation of mitochondrial functions at high Ca+2 levels, as mentioned in the previous chapters. As it is known, at high Ca+2 levels, mitochondria irreversibly lose their ATP synthesis capacity and NAD-dependent respiration abilities. This feature is mainly due to the high membrane potential of mitochondria and the MCU protein (calcium ion carrier protein) properties. The high level of Ca42 in the medium ultimately leads to an increase in permeability at the inner mitochondrial membrane level, and this is known as the permeability transition. Salt and sugar (sucrose, mannitol) groups are added to the incubation medium in order to maintain the osmotic balance. However, with the opening of the pores, the concentration between the medium and the mitochondrial matrix is equalized, resulting in swelling of the mitochondrial structure. The outer membrane, which is shorter than the inner membrane (due to its cristae structure), is broken down first, and this step is followed by the destruction of the inner membrane. As a result of these events, not only the membrane potential decreases, but also NAD/NADPH is lost from the matrix, making oxidation of substrates such as pyruvate and malate impossible. Two possibilities can be considered to explain the survival of mitochondria at high Ca+2 levels: the Ca+2 transporter being blocked or the membrane potential completely destroyed. In a study conducted in 2020, it was shown that the pyruvate and malate oxidizing properties of skeletal muscle mitochondria and its properties for ATP synthesis were lost primarily in standard medium (140 mM Na+, 5 mM K+, and 1 mM Ca+2); it was shared that these properties could be preserved following the blocking of the Ca+2 carrier protein (40).

A second question about the mitochondria transplantation procedure is the continuation of cellular integrity after mitochondria enter the cell. Mc Cully et al., shared that mitochondrias are taken into cells by endocytosis (41). Similar data on the uptake of mitochondria are available in the literature; however, the mechanism for maintaining the integrity of mitochondria after penetration into the cytoplasm remains unclear. To demonstrate the post-transplant robustness of exogenous mitochondria, McCully and colleagues used mitochondria loaded with iron complexes. These complexes were detected in pig heart tissues 4 weeks after transplantation. This result, however, cannot be considered conclusive evidence for mitochondrial robustness. Because it is known that macrophages can maintain iron complexes in phagosomes a few weeks after stem cell administration (42). When in-vitro and in-vivo studies are compared, it is detected that the number of intact mitochondria were found in cardiomyocytes is less in in-vivo studies. When considered together with the data obtained, it is still not clear by which mechanisms the mitochondria, which are few in number, can provide a significant increase in energy production in cardiac muscle cells (3). One view of the efficacy of mitochondrial transplantation is also related to mitochondrial transfer. This situation, in which mitochondria only act as a carrier, is called mitochondrial transformation (43). In some studies in the literature, detection of progression in cardiac functions 10 minutes after mitochondria presentation eliminates the effects of DNA transfer (2, 3, 7).

Another view regarding the method is that after mitochondria transplantation, even autologous, there is a limited immune response in the region. It is known that innate immunity perceives mitochondria and its components as pathogens (44). Studies by Mc Cully et al did not detect an increment in inflammatory markers or proinflammatory cytokine levels. Similarly, no immune response was detected in the rabbit model that was planned with intraperitoneal mitochondria transplantation. However, the local immune response observed in the area of mitochondrial transplantation cannot be ignored according to the literature. In this response, besides neutrophil and macrophage activation in the damaged area, cytokine activation, which affects regional regenerative processes, is important. Along with all the information, the need for experimental data supporting this hypothesis continues (4, 8).

Although there are shared data in the literature on mitochondrial transplantation, the need for evidence for the

transition to clinical applications of the method continues. Experimental animal models in which cardioprotective effects are studied have been studied by a limited team, and the methodological differences between clinical and experimental research models raise questions. While it has been reported that mitochondrial transplantation is performed a few days (up to 15 days) after ischemia in clinical applications; it is found that this time is limited to hours (2 hours) in experimental models (10). Assuming that exogenous mitochondria penetrate cells during the transplantation process, different objections arise regarding the effect of this process on ATP production. The first of these is to ensure the continuation of mitochondrial functions in an environment containing high Ca+2 concentrations. However, the fixation of a very limited number of exogenous mitochondria to the dysfunctional mitochondria structures found in large numbers in the cell is still not clearly explained. Although there is still very limited data in the literature with these processes, the current data still cannot explain these questions clearly. In the light of all the data, although the cardioprotective effects of the existing methods are promising, the need for experimental studies for clinical applications continues (8).

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