EXPERIMENTAL STUDY

Evaluation of the effects of ischemia-reperfusion injury in rat isogenic and allogeneic muscle and skin transplant models

Fatih Ceran,[1] Salih Onur Basat,[2] İdris Ersin,[2] Deniz Filinte,[3] Özgür Pilancı,[2] Mehmet Bozkurt[2]

[1]Department of Plastic, Reconstructive and Aesthetic Surgery, Biruni University Medical Faculty, İstanbul-*Türkiye*
[2]Department of Plastic, Reconstructive and Aesthetic Surgery, Ministry of Health Bagcilar Training & Research Hospital, İstanbul-*Türkiye*
[3]Department of Pathology Marmara University Medical Faculty, İstanbul-*Türkiye*

ABSTRACT

BACKGROUND: Ischemia-reperfusion injury (IRI) is a phenomenon that affects transplant survival. The aim of our study was to examine the effects of IRI in isogenic and allogeneic muscle and skin transplantation models exposed to prolonged warm ischemia.

METHODS: Forty-eight Lewis rats and 16 Brown-Norway rats were used to create four groups: Isogenic Inguinal Flap Transplantation (IST), Isogenic Gastrocnemius Muscle Flap Transplantation (IMT), Allogeneic Inguinal Flap Transplantation (AST), and Allogeneic Gastrocnemius Muscle Flap Transplantation (AMT). Malonyldialdehyde (MDA) and superoxide dismutase (SOD) levels were measured on postoperative days 1, 7, 21, 35, 63, 100, and 120 in all groups. Donor-specific chimerism (DSC) in peripheral blood was evaluated in the allogeneic groups on postoperative days 7, 21, 35, 63, 100, and 120. The microRNA-21 and microRNA-205 levels were evaluated on postoperative days 1, 7, and 120 in all groups. At the end of the study, a histopathological examination was performed.

RESULTS: A statistically significant difference was found between the groups in terms of MDA and SOD levels. DSC was detected in the AMT group. A significant increase in microRNA-205 was observed, especially in the AMT group. There was no significant difference in the number of functional muscle units between the muscle transplantation groups.

CONCLUSION: The presence of DSC in the AMT group and the lack of a significant difference in the number of functional muscle units in the IMT and AMT groups are noteworthy findings.

Keywords: Allogeneic; flap; ischemia-reperfusion injury; isogenic; muscle; skin; transplantation.

INTRODUCTION

The success of composite tissue allotransplantation (CTA) surgery depends mainly on ischemia-reperfusion injury (IRI) and immunological status. Excessive ischemic damage can cause IRI, resulting from energy depletion and the formation of oxidative free radicals.[1,2]

The goal of CTA studies is to promote donor-specific tolerance (DST) and eliminate the negative effects of long-term immunosuppressive protocols.[3] Various procedures have been implemented to induce DST, such as cellular or vascularized bone marrow transplantation and chimeric cell transplantation.[4,5] The objective in all applied protocols is to promote donor-specific chimerism (DSC).[5,6]

MicroRNAs (miRNAs) are non-coding RNA molecules of 21-24 nucleotides in length. Generally, they repress translation or are involved in the degradation of messenger RNA (mRNA).[7] Studies have revealed that some identified miRNAs are closely

Cite this article as: Fatih Ceran F, Basat SO, Ersin İ, Filinte D, Pilancı Ö, Bozkurt M. Evaluation of the effects of ischemia-reperfusion injury in rat isogenic and allogeneic muscle and skin transplant models. Ulus Travma Acil Cerrahi Derg 2024;30:626-634.
Address for correspondence: Fatih Ceran
Biruni University Medical Faculty, İstanbul, Türkiye
E-mail: fatihcrn@hotmail.com
Ulus Travma Acil Cerrahi Derg 2024;30(9):626-634 DOI: 10.14744/tjtes.2024.77415
Submitted: 28.05.2024 Revised: 05.08.2024 Accepted: 06.08.2024 Published: 02.09.2024

associated with ischemia.[8-10]

Malonyldialdehyde (MDA) is a lipid peroxidation product that causes changes in the specific properties of the membrane and disrupts permeability. Superoxide dismutase (SOD) is an endogenous antioxidant that catalyzes the conversion of superoxide free radicals into hydrogen peroxide and molecular oxygen. With IRI, lipid peroxidation products and, in response, antioxidant markers come into play.[11,12]

In our study, we aimed to evaluate the effects of prolonged warm ischemia time and IRI on transplantation in isogenic and allogenic muscle and skin flap transplantation groups biochemically, immunologically, genetically, and histopathologically.

MATERIALS AND METHODS

The study was carried out in the Experimental Research and Skills Training Center Laboratory with the protocol permission numbered 2015/21 and dated 25.01.2016 by the Ethics Committee and the support of the Research and Development Board.

Forty-eight 7-week-old inbred male Lewis rats weighing 150 g and 16 7-week-old inbred male Brown-Norway rats weighing 150 g were used in the study. The rats were divided into four experimental groups:

Group IST: Isogenic Inguinal Flap Transplantation (Recipient: Lewis (RT^{l}) (n=8), Donor: Lewis (n=8) (RT1)), **Group IMT:** Isogenic Gastrocnemius Muscle Flap Transplantation (Recipient: Lewis (n=8) (RT^{l}), Donor: Lewis (n=8) (RT^{l})), **Group AST:** Allogeneic Inguinal Flap Transplantation (Recipient: Lewis (n=8) (RT^{l}, Donor: Brown-Norway (n=8) (LBN, $RT1^{l+n}$), **Group AMT:** Allogeneic Gastrocnemius Muscle Flap Transplantation (Recipient: Lewis (n=8) (RT^{l}), Donor: Brown-Norway (n=8) (LBN, $RT1^{l+n}$)).

All animals were kept in individual cages in a room with standard environmental conditions. The animals were fed ad libidum. Anesthesia was achieved by administering 10 mg/kg ketamine hydrochloride and 1 mg/kg xylazine hydrochloride intramuscularly to the rats. All dissections and anastomoses were performed under a surgical microscope (Zeiss OP-MI 6 SD; Carl Zeiss, Munchen, Germany). Warm ischemia times were applied, taking into account the times obtained from the literature.[13]

Inguinal Flap Preparation and Transplantation

The lines of the 2 x 3 cm elliptical inguinal flap were incised. Vascular structures were cauterized, and the incision continued until the fascia was exposed. After exposing the fascia, the flap edges were fixed with absorbable sutures (4/0 Vicryl; Ethicon Inc., Somerville, NJ, USA). The femoral artery and vein were dissected under the microscope, and a flap based on the femoral vessels was prepared. The flaps were wrapped in gauze soaked in Ringer's crystalloid solution and exposed to warm ischemia (Fig. 1).

For the Isogenic Skin Transplantation (IST) and Allogeneic Skin Transplantation (AST) groups, an oblique incision was made in the inguinal region of the recipient Lewis rats, and the skin and subcutaneous tissues were dissected with a cautery. The dissection plan was expanded, and the femoral artery and vein were accessed with a microscope. Following the ischemia period, the flaps were transplanted by anastomosing the donor and recipient femoral artery and vein end-to-end with a 10/0 nylon suture (Ethicon Inc., Somerville, NJ, USA). The time between the separation of the flap pedicle and the revascularization was 4 hours.

Gastrocnemius Flap Preparation and Transplantation

A boomerang-shaped incision was made in the inguinal region, and the subcutaneous tissues were cut with a cautery. Fascia and muscle layers were dissected appropriately, and the gastrocnemius muscle was exposed. The flap was completely separated over the femoral artery and vein and exposed to warm ischemia by wrapping it in gauze soaked in Ringer's crystalloid solution (Fig. 2).

An oblique incision was made in the inguinal region of the recipient Lewis rats in the Isogenic Muscle Transplantation (IMT) and Allogeneic Muscle Transplantation (AMT) groups, and the skin and subcutaneous tissues were dissected with a cautery. The dissection plan was expanded, and the femoral artery and vein were accessed with a microscope. Following the ischemia period, the flaps were transplanted by anastomosing the recipient and donor femoral artery and vein

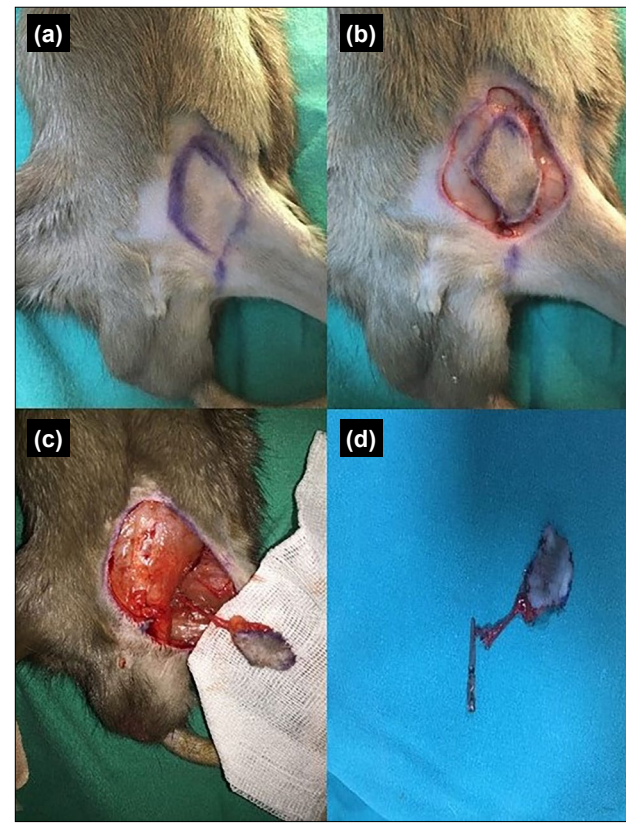

Figure 1. Preparation of the inguinal flap.

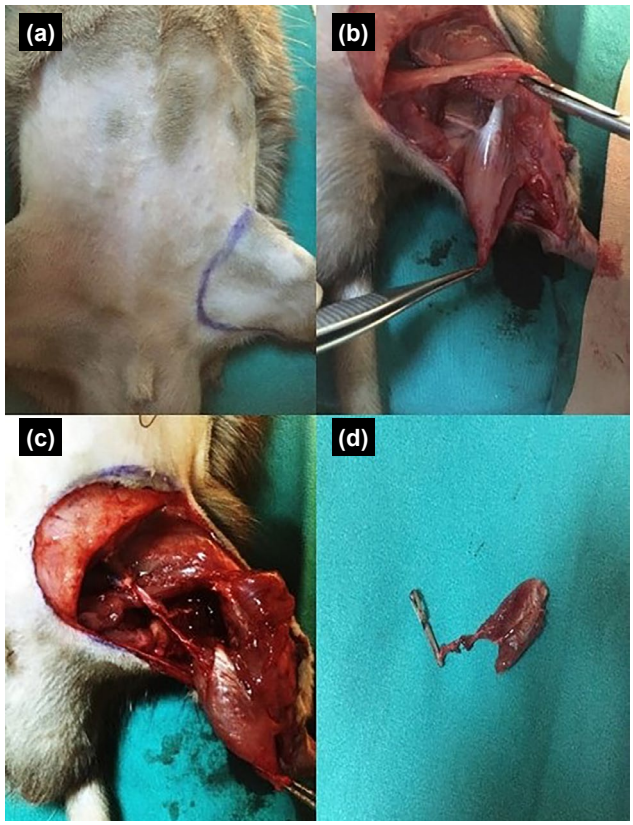

Figure 2. Preparation of the gastrocnemius flap.

end-to-end with a 10/0 nylon suture. The time between the separation of the flap pedicle and the revascularization was 3 hours.

Cyclosporin A (Sandimmun; Novartis Inc., Basel, Switzerland) was administered subcutaneously to rats in the allogeneic groups at a dose of 16 mg/kg/day for immunosuppression.

Examinations Performed at the End of the Study

The immunosuppressive regimen was started at a dose of 16 mg/kg/day, and the dosage was reduced by half weekly. It was continued at a maintenance dose of 2 mg/kg/day until the end of the study.

On the 120th day after the operation, the rats were anesthetized by administering 10 mg/kg ketamine hydrochloride and 1 mg/kg xylazine hydrochloride intramuscularly. The incision lines were incised, and sampled from the anastomosis lines were taken for biochemical, flow cytometric, genetic, and histopathological evaluation.

Biochemical Evaluation

Blood SOD and MDA values were evaluated on postoperative days 1, 7, 21, 35, 63, 100, and 120 in all groups.

The blood sample taken to obtain serum was kept at room temperature for 10-20 minutes, then centrifuged at 2,000-3,000 revolutions per minute (rpm) for 20 minutes, and the supernatant was separated. The obtained serum was placed in an Eppendorf tube and stored at -20 °C.

To evaluate oxidative stress parameters with the Enzyme-Linked Immunosorbent Assay (ELISA) method, serum samples, standards, and controls were placed in micro test wells coated with a monoclonal anti-rat kit antibody. A second biotinylated monoclonal anti-rat antibody was added. After incubation, the unbound antibody was removed by washing. Following this, a streptavidin-horseradish peroxidase enzyme conjugate was added to the wells to ensure binding with the biotinylated antibodies. After the second incubation, the unbound antibody enzyme conjugate in the wells was washed away. It was observed that the solution turned blue when substrate solutions A and B were added. The reaction ended with the addition of an acidic stop solution, and the color turned yellow. After the third incubation, the intensity of the color resulting from enzymatic activity was measured spectrophotometrically at a wavelength of 450 nm. The intensity of the color formed was observed to be directly proportional to the kit concentration present in the sample.

MDA and SOD levels in serum were evaluated using the ELISA method, based on the values specified by the manufacturer (range for MDA: 0.3-65 nmol/ml, sensitivity: 0.208 nmol/ml; range for SOD: 0.5-100 ng/ml, sensitivity: 0.415 ng/ml) (catalog no: 201-11-0157 for MDA, catalog no: 201-02-0169 for SOD; Sunred Co., Shanghai, People's Republic of China).

Determination of Donor-Specific Chimerism in Peripheral Blood by Flow Cytometry

The determination of donor-specific chimerism (DSC) for the Major Histocompatibility Complex (MHC) class I antigen in peripheral blood was performed with a standard two-color flow cytometry device on postoperative days 7, 21, 35, 63, 100, and 120 in the Lewis rats. DSC was analyzed using the mouse anti-rat $RT1^{n\text{-}FITC}$ antibody (for donor MHC class I cells, clone MCA156; Serotec, Kidlington, UK), which was combined with $CD4^{\text{-}PE}$ (clone OX-35), $CD8a^{\text{-}PE}$ (clone OX-8), $CD45RA^{\text{-}PE}$ (clone OX-33), and $CD11_{b/c}^{\text{-}PE}$ (clone OX-42). Following a 30-minute sample incubation period, erythrocytes were lysed, and samples were fixed using 1% paraformaldehyde (PFA) solution. Negative control panels were tested, and samples of isotype-matched antibodies (IgG1-FITC/IgG2-PE) and phosphate-buffered saline were obtained. Analysis was performed on 1×10^4 cells using FACS SCAN (BD Biosciences Pharmingen, NJ, USA) and CellQuest software.

MicroRNA-21 (miRNA) Isolation

miRNA-21 and miRNA-205 were evaluated because they are miRNAs closely related to IRI. miRNA-21 and miRNA-205 levels from blood were evaluated on days 1, 7, and 120 in all groups. miRNA-21 and miRNA-205 levels were measured in biopsy samples or blood from the relevant samples taken at the end of the study. Samples were loaded on agarose gel for quality and quantity determination and spectrophotometric measurement, and each of the different observed bands was loaded into the bioanalyzer. In the samples loaded into the bioanalyzer, 18S and 28S ribosomal RNA (rRNA) peaks were observed as well as regions where small RNAs peaked.

Reverse transcriptase polymerase chain reaction (RT-PCR) was used to quantify miRNA. Target complementary DNA (cDNA) was synthesized from total RNA using reverse transcriptase (RT) primers specific to miRNA. The region of interest was amplified from the obtained cDNAs using miRNA-specific forward primers and universal reverse primers. The RNA samples were diluted to 1 µg in 10 µl. One µl of gene-specific primers and 1 µl of 10 mM deoxynucleotide triphosphate (dNTP) mixture were combined and then incubated at 65°C for 5 minutes. After adding the mixture, the samples were incubated at 42°C for 2 minutes. After adding 1.5 µl of Superscript RT enzyme to each sample, samples were incubated at 42°C for 50 minutes, then at 70°C for 15 minutes. The resulting cDNA samples contained only target miRNA and reference miRNAs, as gene-specific primers were used.

For quantification, Universal Probe Library (UPL) probe 21 and probe 205, which have a complementary sequence in the common sequence region of each miRNA synthesized after RT cDNA synthesis, were used.

After bringing the mixture to a final volume, 8 µ with deionized water (dH2O) was distributed on the plates. Real-time PCR was performed by adding 2 µl (1,000 ng/µl) of cDNA from the test sample to each well that was dispensed with the mixture. Identified miRNAs were studied using the Roche Nano (Roche Inc., NJ, USA) device. Pre-incubation was carried out at 50°C for 2 minutes, incubation at 95°C for 10 minutes, amplification at 95°C for 15 seconds, and 1 minute at 60°C for 40 cycles.

Histopathological Examination

Samples were stained with hematoxylin and eosin (H&E) and desmin dye and examined under a light microscope (Nikon Eclipse 80i; Nikon Instech Co. Ltd., Kawasaki, Kanagawa, Japan). The presence of inflammatory cell infiltration, functional muscle unit count, muscle damage, and necrosis, along with densities, were investigated in the samples. All specimens were evaluated by the same pathologist, and the severity of changes was graded from 0 to 3 (0=absent, 1=mild, 2=moderate, 3=intense).

Statistical Analysis

The Number Cruncher Statistical System 2020 Statistical Software program (NCSS LLC, Kaysville, UT, USA) was used for statistical analysis. Quantitative variables were presented with mean and standard deviation values, and qualitative variables were presented using descriptive statistical methods, such as frequency and percentage. The Shapiro-Wilk test and box plot graphics were used to evaluate the suitability of the data for normal distribution, but due to the number of cases, nonparametric analyses were used.

The Mann–Whitney U test was used to evaluate variables that did not show normal distribution according to two groups. In comparisons of three or more groups, the Kruskal-Wallis test was used, and the Dunn test was used to determine the group causing the difference.

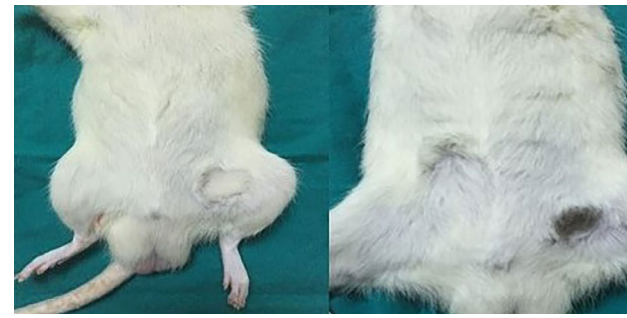

Figure 3. Comparison of isogenic (Left) and allogeneic (Right) inguinal flaps on day 120 post-operation.

The results were evaluated at the 95% confidence interval, and the significance level was $p<0.05$.

RESULTS

After transplantation, all rats were followed up for 4 months. During the 4-month follow-up, there was no rejection in any group (Fig. 3).

Biochemical Evaluation

MDA Levels

A statistically significant difference was found between the groups in the MDA levels on days 1, 7, 21, 35, 63, 100, and 120 ($p=0.001$; $p<0.05$) (Fig. 4).

SOD Levels

A statistically significant difference was found between the SOD levels on days 1, 7, 21, 35, 63, 100, and 120 for the groups ($p=0.001$; $p<0.05$) (Fig. 4).

Donor-Specific Chimerism Results with Flow Cytometry in Peripheral Blood

RT1^n/CD4 Chimerism Level

While the CD4 levels in the AST group on days 7, 21, 35, 63, and 120 were found to be statistically significantly higher than in the AMT group ($p=0.001$; $p<0.05$), the CD4 level on day 100 did not show a statistically significant difference ($p>0.05$) (Fig. 5).

RT1^n/CD8 Chimerism Level

While the CD8 level in the AST group on days 7, 21, 35, 63, and 100 was found to be statistically significantly higher than that of the AMT group ($p=0.001$; $p<0.05$), the CD8 level on day 120 was found to be statistically significantly lower than that of the AMT group ($p=0.001$; $p<0.05$) (Fig. 5).

RT1^n/CD45RA Chimerism Level

CD45RA levels in the AST group on days 7, 21, 35, 63, 100, and 120 were found to be statistically significantly lower than that of the AMT group ($p=0.001$; $p<0.05$) (Fig. 5).

RT1^n/CD11b/c Chimerism Level

CD11$^{b/c}$ levels in the AST group on days 7, 21, 35, and 63

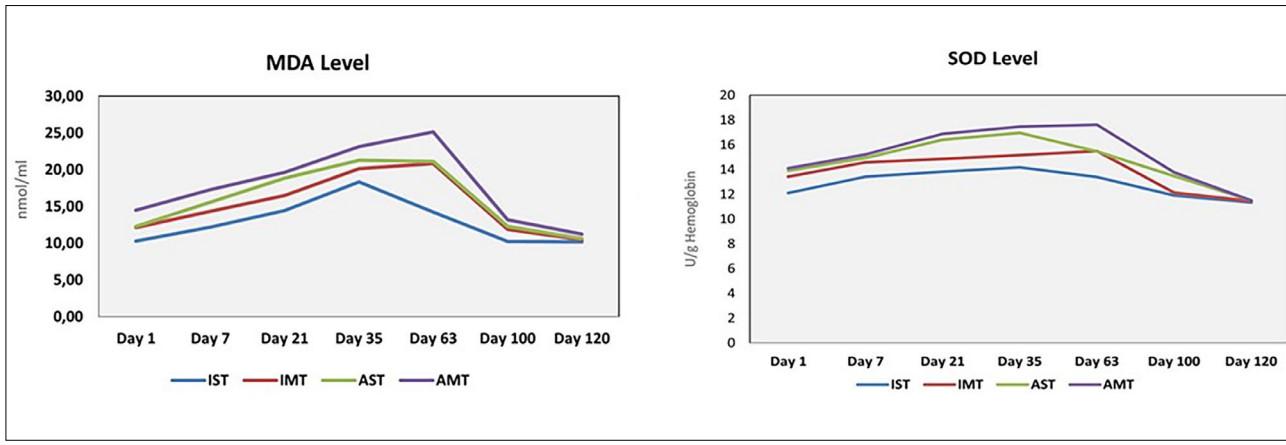

Figure 4. Malondialdehyde (MDA) levels (Left) and Superoxide Dismutase (SOD) levels (Right) by group.

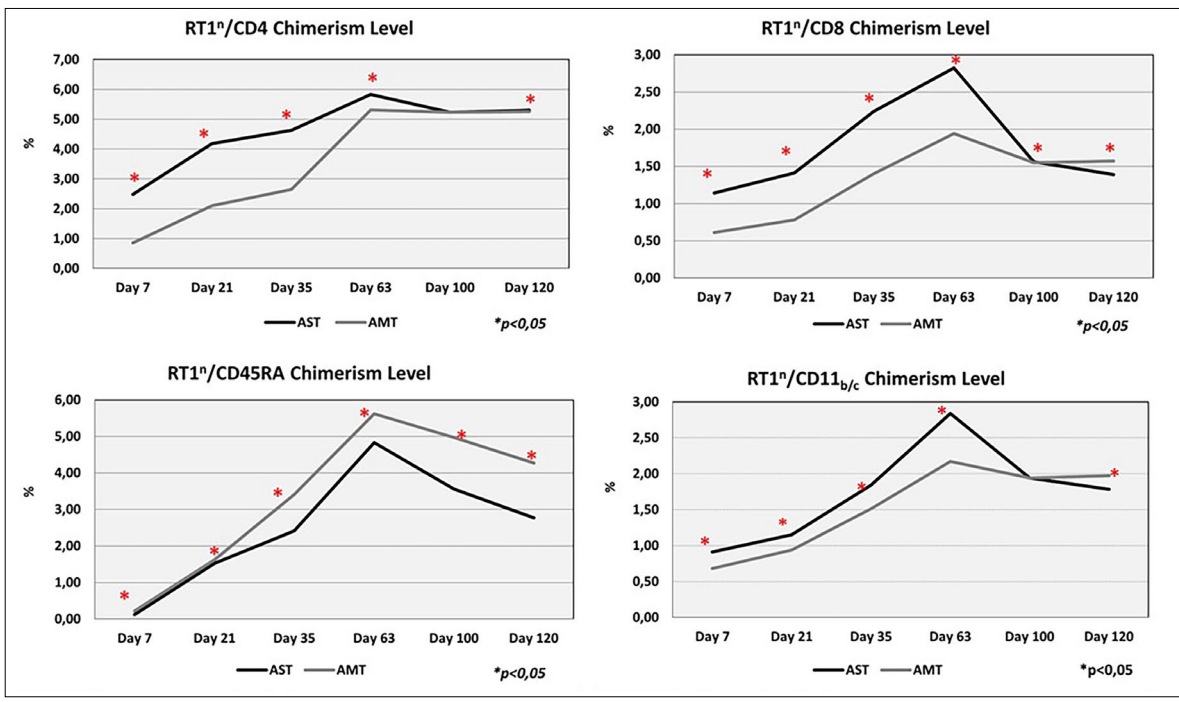

Figure 5. Comparison of CD4, CD8, CD45RA, and CD11b/c levels across groups.

were found to be statistically significantly higher than that of the AMT group (p=0.001; p<0.05). The CD11b/c levels on day 120 in the AST group were found to be statistically significantly lower than that of the AMT group (p=0.001; p<0.05) (Fig. 5).

miRNA-21 and miRNA-205 Levels

miRNA-21 Assessment

A statistically significant difference was found between the miRNA-21 values on days 1, 7, and 120 between the groups (p=0.001; p<0.05) (Fig. 6).

MiRNA-205 Assessment

A statistically significant difference was found between the miRNA-205 values on days 1, 7, and 120 between the groups (p=0.001; p<0.05) (Fig. 6).

Histopathological Findings

Compared to other groups, there was statistically significant inflammatory cell infiltration in both the AST group (p<0.05) (Fig. 7A-B) and AMT group (p<0.05) (Fig. 7C-D).

Intense fibrosis and atrophy were observed in muscle samples. There was no statistically significant difference between the two groups in terms of muscle damage (p>0.05) (Fig. 7E-F). As a result of desmin staining performed to compare the functional muscle units in the isogenic and allogenic groups, no statistically significant difference was observed in the number of functional muscle units in either the IMT group or the AMT group (p>0.05) (Fig. 7G-H).

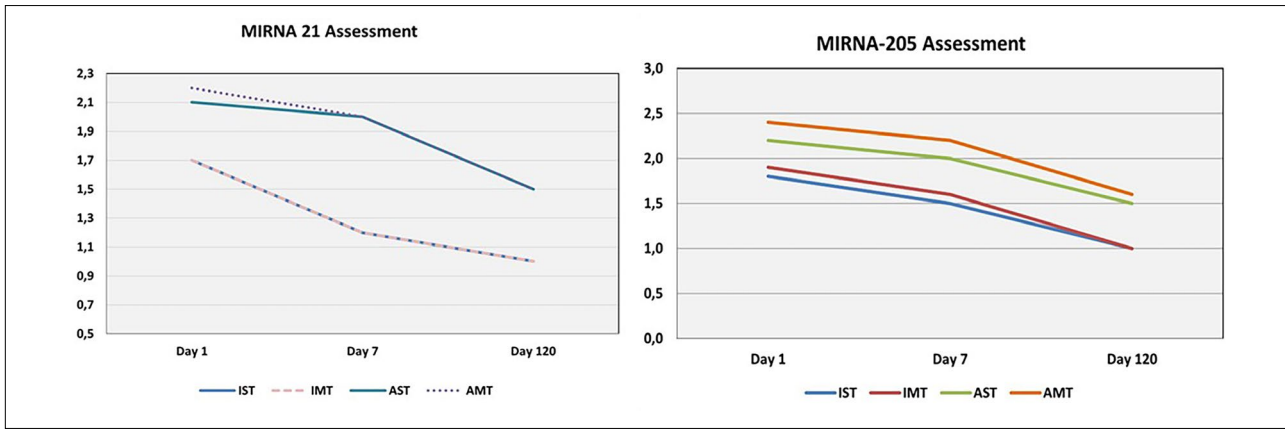

Figure 6. Levels of microRNA (miRNA-21) (Left) and miRNA-205 (Right) across groups.

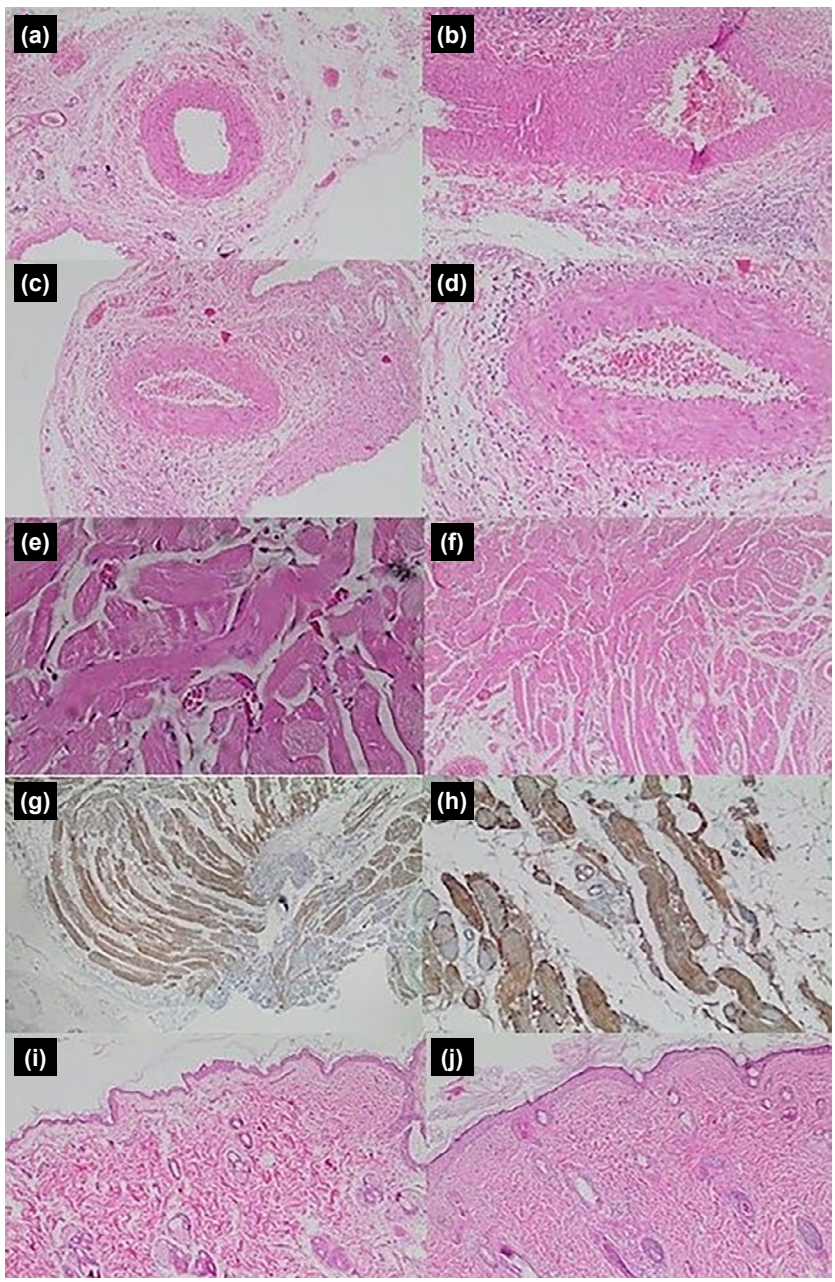

Figure 6. (a) Mild perivascular inflammatory cell infiltration in isogenic inguinal flap (x100). **(b)** Intense perivascular inflammatory cell infiltration in allogeneic inguinal flap (x100). **(c)** Mild perivascular inflammatory cell infiltration in isogenic gastrocnemius flap transplantation group (x100). **(d)** Significant perivascular inflammatory cell infiltration in allogeneic gastrocnemius flap transplantation group (x200). **(e)** No significant difference in inflammatory cell infiltration between isogenic (x400) and **(f)** allogeneic gastrocnemius flap transplantation groups (x100). **(g)** Desmin staining to assess functional muscle units in the isogenic gastrocnemius flap transplantation group (x40). **(h)** Desmin staining in the allogeneic gastrocnemius flap transplantation group (x200). **(i)** Mild inflammatory cell infiltration of the skin in the isogenic inguinal flap transplantation group (x100). **(j)** Significant inflammatory cell infiltration in the skin of the allogeneic inguinal flap transplantation group (x100).

Table 1. Evaluation of pathology results by group

	IST	IMT	AST	AMT	p
Inflamatory Cell Infiltration	8	8	0	0	0.001**
	0	0	8	8	
Inflamatory Cell Infiltration to Skin	8	–	0	–	0.001**
	0	–	8	–	
Muscle Damage	–	8	–	8	–
Functional Unit Number	–	8	–	8	–

**p<0.01

There was statistically significant inflammatory cell infiltration in the AST group (p<0.05) (Fig. 7I-J) (Table 1).

DISCUSSION

Although studies suggest that flap failure may be caused by prolonged ischemia-reperfusion (I/R) time, most of the studies investigating the relationship between ischemia time and rejection of transplanted organs have been unsuccessful.[14-17] Ischemia times for human muscle and skin under normothermic conditions have been described in the literature.[13] In this study, we exposed skin flaps to 4 hours of warm ischemia and muscle flaps to 3 hours of warm ischemia, taking into account the warm ischemia times given in the literature. The ultimate purpose of choosing these periods was to objectively reveal all the effects of I/R damage.

Free oxygen radicals cause lipid peroxidation and epithelial damage, along with neutrophil activation. MDA and SOD are important markers of lipid peroxidation and antioxidant defense.[18] Hassan et al. studied these biomarkers at preoperative hours 0, 1, 6, 12, 24, 48, and 72, and days 5 and 7, finding that MDA and SOD levels increased in a correlational manner postoperatively.[19]

We evaluated SOD and MDA values in all groups on days 1, 7, 21, 35, 63, 100, and 120 and objectively determined the detailed calendar amounts of these biochemical components after the transplantation process. Our results are in line with the literature. However, our study contributes to the literature in terms of revealing long-term results on a calendar basis.

If the immunological cascade is used correctly, DST can be developed in the recipient. As a result of tolerance development, the necessity of using immunosuppressive agents is eliminated, and morbidities that may occur due to the use of these suppressive agents can be eliminated.[20-22] Chimerism undoubtedly plays a very important role in the development of tolerance. Chimerism basically means the appearance of donor cells in the recipient. In large-scale studies on tolerance induction conducted in the last two decades, donor-specific chimeric formation that may accompany tolerance induction in CTA has been evaluated in detail.[23-25] Siemionow et al., in their study, pointed out that DSC occurred in vascularized skin grafts as a result of low-dose cyclosporine treatment and that tolerance induction developed later on.[26] In the investigation of minimal immunosuppressive and tolerance-inducing protocols, researchers evaluated the role of DSC in CTA acceptance and survival.[1,26] The role of chimerism in prolonging the survival of organ allografts has been documented, but there are also reports showing a lack of correlation between chimerism and tolerance.[26-30] Although there are no studies in the literature on chimerism induction as a result of muscle transplantation, chimerism induction has been documented in experimental lower extremity transplantation models.[26,31] However, in lower extremity transplantation, tissues such as bone marrow and skin are extremely immunogenic, and there is no evidence of muscle tissue chimerism induction.

Chimerism was demonstrated in the inguinal flap group, as stated in the literature. Likewise, we demonstrated the induction of chimerism in the allogeneic muscle flap. Documenting the induction of chimerism in muscle tissue can be considered a contribution to the literature. However, it should not be overlooked that no human studies have demonstrated significant and durable DSC, and, to date, there have been no recommendations regarding the feasibility of tolerance induction based on clinical results.

miRNAs bind to mRNAs that are complementary to their own nucleotide sequences and regulate post-transcriptional gene expression by translational repression or mRNA degradation.[32,33] Impaired miRNA expression levels have been demonstrated in transient focal ischemic attack and myocardial IR injury, and the cardioprotective effect of endogenously synthesized miRNAs in IR has been demonstrated.[7-10] A group of miRNAs has been shown to be effective in skeletal muscle development, restructuring, and even myopathies. In their study, Hsieh et al. showed that miRNA-21, miRNA-200, and miRNA-205 were upregulated in the gracilis muscle after ischemia.[34] Likewise, it has been shown in various publications that a group of miRNAs are upregulated as a result of skin flap ischemia.[35]

Due to previous studies conducted on muscle and skin mod-

els, we also measured miRNA-21 and miRNA-205 levels in our study. The results of the study are parallel to the literature in terms of miRNA levels. One of the advantages of our study is that the experimental values are revealed gradually. miRNA-21 and miRNA-205 levels increased as a result of I/R in both flap groups.

Inflammatory cell infiltration, skin necrosis, and muscle damage are some of the histopathological steps and consequences.[26,30,31] As expected, more inflammatory cell infiltration was observed in the allogeneic groups. Cell infiltration, especially in the perivascular area, may cause the anastomosis line to malfunction. In previous studies, it was stated that the transplanted muscle-free flaps could functio.[36] However, there is no detailed study on the functional units and histopathology of the transplanted muscle. Remarkably, as a result of desmin staining, no statistically significant difference was observed in the number of functional muscle units in the isogenic and allogeneic gastrocnemius flap transplantation groups, and this finding is a new contribution to the literature.

CONCLUSION

No difference in the number of functional muscle units between isogenic and allogeneic muscle transplantation groups and an increase of miRNA-205 in muscle transplantations, high levels of chimerism in skin transplantations, and the presence of chimerism in the muscle transplantation model are remarkable findings in this study. However, future research is needed to advance these findings.

Ethics Committee Approval: This study was approved by the Ministry of Health Bagcilar Training & Research Hospital Ethics Committee (Date: 25.01.2016, Decision No: 2015-21).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: F.C., S.O.B., İ.E., D.F., Ö.P., M.B.; Design: F.C., S.O.B., İ.E., D.F., Ö.P., M.B.; Supervision: F.C., S.O.B., İ.E., D.F., Ö.P., M.B.; Resource: F.C., S.O.B., İ.E., D.F., Ö.P., M.B.; Materials: F.C., S.O.B., İ.E., D.F., Ö.P., M.B.; Data collection and/or processing: F.C., S.O.B., İ.E., D.F., Ö.P., M.B.; Analysis and/or interpretation: F.C., S.O.B., İ.E., D.F., Ö.P., M.B.; Literature search: F.C., S.O.B., İ.E., D.F., Ö.P., M.B.; Writing: F.C., S.O.B., İ.E., D.F., Ö.P., M.B.; Critical reviews: F.C., S.O.B., İ.E., D.F., Ö.P., M.B.

Conflict of Interest: None declared.

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

REFERENCES

1. Siemionow M, Agaoglu G. Tissue transplantation in plastic surgery. Clin Plast Surg 2007;34:251–ix. [CrossRef]
2. Ravindra KV, Wu S, Bozulic L, Xu H, Breidenbach WC, Ildstad ST. Composite tissue transplantation: a rapidly advancing field. Transplant Proc 2008;40:1237–48. [CrossRef]
3. Horner BM, Randolph MA, Huang CA, Butler PE. Skin tolerance: In search of the holy grail. Transpl Int 2008;21:101–12.
4. Kulahci Y, Altuntas SH, Karagoz H, Cwykiel JM, Zor F, Siemionow M. A new total hemiface allotransplantation model in rats. Microsurgery 2016;36:230–8. [CrossRef]
5. Siemionow M, Rampazzo A, Gharb BB, Cwykiel J, Klimczak A, Madajka M, et al. The reversed paradigm of chimerism induction: Donor conditioning with recipient-derived bone marrow cells as a novel approach for tolerance induction in vascularized composite allotransplantation. Microsurgery 2016;36:676–83. [CrossRef]
6. Hequet O, Morelon E, Bourgeot JP, Dubois V, Devauchelle B, Dubernard JM, Michallet M. Allogeneic donor bone marrow cells recovery and infusion after allogeneic face transplantation from the same donor. Bone Marrow Transplant 2008;41:1059–61. [CrossRef]
7. Banerjee J, Sen CK. MicroRNAs in skin and wound healing. Methods Mol Biol 2013;936:343–56. [CrossRef]
8. Loscalzo J. The cellular response to hypoxia: tuning the system with microRNAs. J Clin Invest 2010;120:3815–7. [CrossRef]
9. Crosby ME, Kulshreshtha R, Ivan M, Glazer PM. MicroRNA regulation of DNA repair gene expression in hypoxic stress. Cancer Res 2009;69:1221–9. [CrossRef]
10. Weiss JB, Eisenhardt SU, Stark GB, Bode C, Moser M, Grundmann S. MicroRNAs in ischemia-reperfusion injury. Am J Cardiovasc Dis 2012;2:237–47.
11. McCord JM. Oxygen-derived radicals: a link between reperfusion injury and inflammation. Fed Proc 1987;46:2402–6.
12. Korthius RJ, Granger DN. Ischemia-reperfusion injury: Role of oxygen-derived free radicals. In: Taylor AE, Matalon S, Ward PA, editors. Physiology of Oxygen Radicals. Bethesda, Maryland: American Physiological Society; 1986.p.217.
13. Blaisdell FW. The pathophysiology of skeletal muscle ischemia and the reperfusion syndrome: a review. Cardiovasc Surg 2002;10:620–30.
14. Lee WP, Pan YC, Kesmarky S, Randolph MA, Fiala TS, Amarante MT, Weiland AJ, Yaremchuk MJ. Experimental orthotopic transplantation of vascularized skeletal allografts: functional assessment and long-term survival. Plast Reconstr Surg 1995;95:336–49; discussion 350–3. [CrossRef]
15. Büttemeyer R, Jones NF, Min Z, Rao U. Rejection of the component tissues of limb allografts in rats immunosuppressed with FK-506 and cyclosporine. Plast Reconstr Surg 1996;97:139–51. [CrossRef]
16. Mathes SJ. Flap Physiology. Mathes Plastic Surgery. In: Hentz VR, editor. Philadelphia: WB Saunders; 2006.p.483–506.
17. Lee WP. Composite tissue transplantation: more science and patience needed. Plast Reconstr Surg 2001;107:1066–70. [CrossRef]
18. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 1985;312:159–63. [CrossRef]
19. Hassan L, Bueno P, Ferrón-Celma I, Ramia JM, Garrote D, Muffak K, et al. Time course of antioxidant enzyme activities in liver transplant recipients. Transplant Proc 2005;37:3932–5. [CrossRef]
20. Hughes AL, Yeager M. Molecular evolution of the vertebrate immune system. Bioessays 1997;19:777–86. [CrossRef]
21. Moskophidis D, Laine E, Zinkernagel RM. Peripheral clonal deletion of antiviral memory CD8+ T cells. Eur J Immunol 1993;23:3306–11.
22. Schwartz RH. Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. Cell 1992;71:1065–8. [CrossRef]
23. Yin D, Fathman CG. CD4-positive suppressor cells block allotransplant rejection. J Immunol 1995;154:6339–45. [CrossRef]
24. Sharabi Y, Sachs DH. Mixed chimerism and permanent specific transplantation tolerance induced by a nonlethal preparative regimen. J Exp Med 1989;169:493–502. [CrossRef]
25. Sykes M. Immune tolerance: mechanisms and application in clinical transplantation. J Intern Med 2007;262:288–310. [CrossRef]
26. Siemionow MZ, Izycki DM, Zielinski M. Donor-specific tolerance in fully major histocompatibility major histocompatibility complex-mismatched limb allograft transplants under an anti-alphabeta T-cell recep-

tor monoclonal antibody and cyclosporine A protocol. Transplantation 2003;76:1662–8. [CrossRef]
27. Ozer K, Oke R, Gurunluoglu R, Zielinski M, Izycki D, Prajapati R, Siemionow M. Induction of tolerance to hind limb allografts in rats receiving cyclosporine A and antilymphocyte serum: effect of duration of the treatment. Transplantation 2003;75:31–6. [CrossRef]
28. Lee WP, Butler PE, Randolph MA, Yaremchuk MJ. Donor modification leads to prolonged survival of limb allografts. Plast Reconstr Surg 2001;108:1235–41. [CrossRef]
29. Demir Y, Ozmen S, Klimczak A, Mukherjee AL, Siemionow MZ. Strategies to develop chimerism in vascularized skin allografts across MHC barrier. Microsurgery 2005;25:415–22. [CrossRef]
30. Nasir S, Bozkurt M, Krokowicz L, Klimczak A, Siemionow M. Correlation of chimerism with graft size and revascularization in vascularized and nonvascularized skin allografts. Ann Plast Surg 2009;62:430–8.
31. Gordon CR, Nazzal J, Lozano-Calderan SA, Lee SG, Lee WP, Siemionow M, et al. From experimental rat hindlimb to clinical face composite tissue allotransplantation: historical background and current status. Microsurgery 2006;26:566–72. [CrossRef]
32. Broderick JA, Zamore PD. MicroRNA therapeutics. Gene Ther 2011;18:1104–10. [CrossRef]
33. Pasquinelli AE. Molecular biology. Paring miRNAs through pairing. Science 2010;328:1494–5. [CrossRef]
34. Hsieh CH, Jeng JC, Jeng SF, Wu CJ, Lu TH, Liliang PC, et al. MicroRNA profiling in ischemic injury of the gracilis muscle in rats. BMC Musculoskelet Disord 2010;11:123. [CrossRef]
35. Sen CK, Gordillo GM, Khanna S, Roy S. Micromanaging vascular biology: tiny microRNAs play big band. J Vasc Res 2009;46:527–40. [CrossRef]
36. Terzis JK, Sweet RC, Dykes RW, Williams HB. Recovery of function in free muscle transplants using microneurovascular anastomoses. J Hand Surg Am 1978;3:37–59. [CrossRef]

DENEYSEL ÇALIŞMA - *ÖZ*

Sıçan izojenik ve allojenik kas ve deri transplantasyon modellerinde iskemi-reperfüzyon hasarının etkilerinin değerlendirilmesi

Fatih Ceran,[1] Salih Onur Basat,[2] İdris Ersin,[2] Deniz Filinte,[3] Özgür Pilancı,[2] Mehmet Bozkurt[2]

[1]Biruni Üniversitesi Tıp Fakültesi, Plastik, Rekonstrüktif ve Estetik Cerrahi Anabilim Dalı, İstanbul, Türkiye
[2]Sağlık Bakanlığı Bağcılar Eğitim ve Araştırma Hastanesi, Plastik, Rekonstrüktif ve Estetik Cerrahi Anabilim Dalı, İstanbul, Türkiye
[3]Marmara Üniversitesi Tıp Fakültesi, Patoloji Anabilim Dalı, İstanbul, Türkiye

AMAÇ: İskemi-reperfüzyon hasarı, transplantasyon başarısını etkileyen bir durumdur. Çalışmamızın amacı, uzun süreli sıcak iskemiye maruz kalan izojenik ve allojenik kas ve deri transplantasyon modellerinde iskemi-reperfüzyon hasarının etkilerini incelemektir.
GEREÇ VE YÖNTEM: 48 Lewis ve 16 Brown-Norway sıçan dört grup oluşturmak üzere kullanıldı: IST: İzojenik inguinal flep transplantasyonu, IMT: İzojenik gastroknemius kas flebi transplantasyonu, AST: Allojenik inguinal flep transplantasyonu ve AMT: Allojenik gastroknemius kas flebi transplantasyonu. Tüm gruplarda postoperatif 1., 7., 21., 35., 63., 100. ve 120. günlerde malondialdehit (MDA) ve süperoksit dismutaz (SOD) değerleri ölçüldü. Allojenik gruplarda postoperatif 7., 21., 35., 63., 100. ve 120. günlerde periferik kanda donör spesifik kimerizm (DSC) değerlendirildi. Tüm gruplarda postoperatif 1., 7. ve 120. günlerde miRNA-21 ve miRNA-205 düzeyleri değerlendirildi. Çalışmanın sonunda histopatolojik çalışma yapıldı.
BULGULAR: Gruplar arasında MDA ve SOD değerleri açısından istatistiksel olarak anlamlı fark bulundu. AMT grubunda DSC tespit edildi. Özellikle AMT grubunda miRNA-205'te anlamlı artış gözlendi. Kas transplantasyon grupları arasında fonksiyonel kas ünitesi sayısı açısından anlamlı bir fark yoktu.
SONUÇ: AMT grubunda DSC varlığı ve IMT ve AMT gruplarında fonksiyonel kas ünitesi sayısı arasında anlamlı bir fark olmaması dikkat çekici bulgulardır.

Anahtar sözcükler: Allojenik; deri; flep; iskemi-reperfüzyon hasarı; izojenik; kas; transplantasyon.

Ulus Travma Acil Cerrahi Derg 2024;30(9):626-634 DOI: 10.14744/tjtes.2024.77415