Research Article

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Effects of mesenchymal stem cell and amnion membrane transfer on prevention of pericardial adhesions

Mezenkimal kök hücre ve amniyon membran transferinin perikardial adezyonların önlenmesi üzerine etkileri

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Abstract

Background: To investigate and compare the antiadhesive/antifibrotic effects of mesenchymal stem cells (MSC) and amnion membrane transfer (AMT) in a rat model.

Material and methods: Three experimental and sham groups were formed using 30 Wistar-Albino rats. AMT and MSC were applied to the related groups. The control group was not treated. After 12 weeks follow-up, intracardiac blood and cardiac-pericardiac tissue samples were taken. The severity of adhesions and fibrosis were scored macroscopically and microscopically with Hematoxylin/

Eosin and Masson's trichrome staining. TNF- α , TGF- β , IL-1, PDGF, FGF, VEGF and Caspase-3 levels were measured with the ELISA method.

Results: Severe adhesions were observed in the AMT and control groups, but no adhesion was present in the MSC group. Pericardial thickness, increased vascularity, fibrosis, and collagen accumulation were similar between control and AMT groups, but were less in Sham and MSC groups. Between MSC and AMT groups, only Caspase-3 level was different, which is an apoptosis marker.

Conclusion: The positive effects of MSC on adhesion, which we achieved in our study, suggest that it may prevent adhesion. AMT did not provide a positive effect. The correlation of Caspase-3 with postoperative adhesion/fibrosis should be examined in more detail.

Keywords: Mesenchymal stem cell; Amnion membrane transfer; Pericardial adhesion; Rat; Inflammatory markers.

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Öz

Amaç: Mezenkimal kök hücrelerin (MSC) ve amnion membran transferinin (AMT) bir sıçan modelinde antiadhezif/antifibrotik etkilerini araştırmak ve karşılaştırmak.

Gereç ve Yöntem: Otuz adet Wistar albino sıçan ile bir şem grubu ve üç deney grubu oluşturuldu. İlgili gruplara AMT ve MSC uygulandı. Kontrol grubuna tedavi verilmedi. 12 haftalık takipten sonra intrakardiyak-kan ve kardiyak-perikardiyal doku örnekleri alındı. Adezyonların ve fibrozun şiddeti makroskopik ve mikroskopik olarak Hematoksilin/Eozin ve Masson trikrom boyanması

ile skorlandı. TNF- α , TGF- β , IL-1, PDGF, FGF, VEGF ve Kaspaz-3 seviyeleri ELISA yöntemi ile ölçüldü.

Bulgular: AMT ve kontrol gruplarında şiddetli adezyon gözlemlendi, ancak MSC grubunda adezyon yoktu. Perikard kalınlığı, artmış vaskülarite, fibrozis ve kollajen birikimi kontrol ve AMT grupları arasında benzerdi ancak Sham ve MSC grupları ile daha az benzerdi. MSC ve AMT grupları arasında sadece bir apoptoz belirteci olan Kaspaz-3 seviyesi farklıydı.

Sonuç: Çalışmamızda elde ettiğimiz MSC'nin adezyon üzerindeki olumlu etkileri, adezyonu önleyebileceğini düşündürmektedir. AMT olumlu bir etki sağlamadı. Caspase-3'ün postoperatif adezyon/fibroz ile korelasyonu daha ayrıntılı olarak incelenmelidir.

Anahtar Kelimeler: Mezenkimal kök hücre; Amniyon membran transferi; Perikardiyal adezyon; Sıçan; İnflamatuvar belirteçler.

Introduction

In cardiovascular surgery, adhesions occur between the heart and pericardial tissue after pericardiotomy, and these adhesions increase the risk of cardiac and major vessel injury in recurrent surgical procedures [1, 2]. Depending on the procedures performed in cardiovascular surgery, the pericardial structure is not the same as in the pre-operative period. During surgery, pericardial tissue is exposed to various tensile forces, friction, abrasion, increasing edema and most importantly long-term contact with air and blood. This exposure causes serious adhesions in the surgical area in the short- and medium-term [3]. In order to reduce these adhesions, many methods are used, such as covering part of the pericardium just above the large vessels, using different synthetic and biological grafts, bioabsorbable membranes (films) and systemic anti-inflammatory agents [4]. Two biological methods have been proposed for the treatment of damaged tissues and organs. The amnion membrane transfer (AMT) method was described in 1910 [5]. The mesenchymal stem cell (MSC) application, which has similar results to AMT, is a remarkable topic in recent years [6]. The anti-inflammatory, antifibrotic, antiproliferative, angiogenic and immunomodulatory properties of MSCs are known [7]. The easiest type is derived from adipose tissue because it is isolated from fat cells collected by liposuction [8]. MSCs, according to the conditions, play a positive regulatory role as well as having an important negative role, thus stopping the cytokine storm in inflammation [9]. Amniotic membrane (AM), which includes

stem cells containing extracellular matrix, is a translucent material with anti-inflammatory, antifibrotic, and anti-scarring effects that contributes to wound healing [10]. Similar to fetal wound healing without scars, it was reported that a high anti-scarring effect could be obtained with various factors carried by the amniotic membrane. It is thought that the potential adhesive tissue layer prevents surface adhesion and fibrosis by acting as a physical barrier [11]. AM has anti-inflammatory and antibacterial effects. More importantly, AM does not have HLA-B-DR histocompatibility antigens and because of this immunological advantage, there is no need for donor compatibility and thus the problem of rejection does not occur in tissues [12]. AM contains a certain amount of stem cells [13], but at the same time, these stem cells do not cause biocompatibility because of their low immunogenic and low antigenic properties [14].

This study was designed to comparatively evaluate the effect of AMT and MSC methods that can be used to reduce postoperative pericardial adhesion/fibrosis and inflammation.

Materials and methods

This study was performed with the approval of the Ethics Committee for Local Animal Research (approval number: 16/52, date 02/05/2016). All animals were treated in accordance with the guidelines of the Guide to the Care and Use of Laboratory Animals published by the National Academy of Sciences. All surgical procedures, animal care and follow-up were performed at the Veterinary Faculty, Kırıkkale University (KU).

Animal model

We included 30 male Wistar Albino rats. The animals were randomly divided into three treatment groups and a sham group; MSC (Group M, n=9), AMT (Group A, n=9), control (Group C, n=9) and sham (Group S, n=3). The aim of incorporating the sham group into the present study is to compare the success of one or both treatment methods with no pericardiotomy performed group (Sham) histopathologically and macroscopically. Maximum five rats were housed in the cages during the experiments. Rats were housed in a controlled environment (12 h light/darkness cycle, temperature; $\sim 24 \pm 2^\circ\text{C}$, humidity; $\sim 50 \pm 7\%$). Rats were fed with standard food and tap water ad libitum. All groups were followed for 12 weeks.

Surgical technique

Anesthesia: The rats were injected with 100 mg/mL intramuscular (IM) dose of ketamine hydrochloride 2:1 (v/v) (Pfizer, Luleburgaz, Turkey) and a 23.3 mg/mL IM dose of xylazine hydrochloride (Intermed, Ankara, Turkey) (total 0.75 mL/kg IM) for anesthesia. Then 0.2 mg/kg butorphanol (Butomidor, Interhas, Turkey) was used for analgesia in 2-h intervals. The thorax and neck areas of the rats were stained with antiseptic povidone iodine (Batticon, Adeka, Istanbul, Turkey) after shaving. After tracheostomy, rats were intubated by using a 20-G, 32 mm pink Teflon iv. Cannula (Novacath, Çorum, Turkey). The animals were mechanically ventilated with an oxygen-air mixture of 1.5-2 L/min at 20–30 breaths/min.

Pericardiotomy

Paramedian sternotomy and pericardiotomy were performed by cutting the 3rd, 4th and 5th left hemithorax costal cartilages. Massive hemorrhage due to internal mammarian artery injury were controlled by suturing and cauterization. For 15 min, all rats were exposed to air and blood that collected around the pericardium. No protective intervention was applied to rats in the Group-C. Amniotic membranes were obtained from other rats spontaneously delivered by the natural route. After these biological materials were completely cleaned with saline, they were placed in the pericardial area of the rats in the AMT group on the same day. Green fluorescent protein (GFP)-labeled rat adipose-derived mesenchymal stem cells (RaMSCs) (MLP Laboratory, Istanbul, Turkey), which are commercially available, were applied to the MSC group as 2×10^6 cells. When the anterior thoracic wall was closed with sutures, stem cells which can be applied onto heart and have adhesive properties [15] for the surface were confined to the left thorax-mediastinum cavity. In order not to affect the tissue response, a suture was not used to pericardial tissue in any groups. After pericardial intervention, the thorax was closed under positive ventilation pressure to avoid pneumothorax, with rapid subcutaneous suture with 3/0 poliglecaprone 25 (Monocryl®, Ethicon, San Lorenzo, Puerto Rico) while the entire lung was inflated. Then the skin was closed with 3/0 polypropylene suture (Propylene, Dogsan, Trabzon, Turkey). Ventilator support continued until spontaneous breathing was stabilized. The ventilator was deactivated before the weaning phase. After ensuring that respiration and hemodynamics were not impaired, the tracheal cannula was removed and extubated. Then the skin and

subcutaneous tissues in the neck area were sutured. In the maintenance unit providing oxygen, heating and humidity support, rats were followed for 2 h. Then, the animals were taken to their cages.

Reoperation

The rats were followed for 12-weeks after the first operation and then reoperated. All rats were anesthetized as previously described. At least 5 mL of the intracardiac blood sample was taken from each rat and sacrifice was completed. Paramedian sternotomy was performed by cutting the left hemithorax costal cartilages. Wide exposure of the entire thorax was provided to visualize and evaluate the adhesion better. Adhesions between visceral and parietal pericardium were macroscopically scored according to the peritoneal adhesion index and photographed. The severity of adhesions was graded 0: No adhesions; 1: Slim and easily separable adhesions; 2: Moderate adhesions with blunt dissection; 3: Severe adhesions necessitating sharp dissection following Coccolini et al. [16].

Cardiac-pericardiac tissue specimens collected for advanced analysis were fixed in formalin (10%), embedded in paraffin blocks, stained with Hematoxylin/Eosin (H/E) and Masson's trichrome (MST).

Histopathologic scoring of tissue repair response was graded for fibrosis, histiocytosis, vascularisation, granulocytic response. For fibrosis, 0: no; 1: few fibroblasts; 2: fibroblastic proliferation and increased collagen; 3: fibrosis, collagen bundles. For histiocytosis, 0: no; 1: rare macrophage; 2: high amounts of histiocytes, rare multinucleated giant cells; 3: granuloma formation. For vascularisation, 0: no; 1: mild vasodilatation; 2: severe congestion; 3: hemorrhage, neovascularization. For granulocytic response, 0: no; 1: rare macrophage; 2: high amounts of histiocytes, rare multinucleated giant cells; 3: granuloma formation as scored.

Inflammatory cell reaction was scored in HE and MST stained sections at X20 magnification (Nikon Eclipse E600, 0.785 mm², Nikon AG Instruments, Switzerland). Overall inflammatory reaction and the amount of inflammation, fibrosis, vascularization and granulocytic responses were assessed by using a four-degree scale. A score of 0 indicated an absence of reaction, 1 was weak, 2 was moderate, and 3 was severe reaction. A score of 0 was given when there was no increase in these parameters, and 1 denoted mild and patchy increase. A score of 2 was given when these parameters formed a prominent reaction, and a score of 3 denoted band-like infiltrate with some destruction of normal structures.

Pericardial thickness was evaluated by staining the pericardial tissue samples obtained from the lateral surface of the heart with H/E and MST. Collagen deposition and fibrosis were also evaluated by MST staining

IFA (immunofluorescent-antibody) imaging: GFP labeled stem cells were visualized and recorded with a fluorescent antibody microscope.

TGF- β (transforming growth factor beta), IL-1 (interleukin-1), PDGF (platelet derived growth factor), FGF (fibroblast growth factor), VEGF (vascular endothelial growth factor) and non-cleaved Caspase-3 levels were measured in the intracardiac blood samples using an ELISA kit-Rat form (ELABSCIENCE, Wuhan, Hubei, China). The collected blood was centrifuged at 4000 rpm for 10 min and serum samples were obtained. These samples were used in ELISA method.

Performance characteristics of the ELISA kit assays

On the day of analysis, sera were allowed to dissolve at room temperature. The analytical (linear) measurement ranges for FGF, TGF- β , IL-1, VEGF, TNF- α , PDGF and Caspase-3 were 15.63–1000 pg/mL, 31.25–2000 pg/mL, 31.25–2000 pg/mL, 31.25–2000 pg/mL, 78.13–5000 pg/mL, 31.25–2000 pg/mL and 0.31–20.0 ng/mL, respectively. The minimal detection limit for FGF, TGF- β , IL-1, VEGF, TNF- α , PDGF and Caspase-3 were 9.38 pg/mL, 18.75 pg/mL, 18.75 pg/mL, 18.75 pg/mL, 46.88 pg/mL, 18.75 pg/mL and 0.19 ng/mL, respectively. The reported intraassay variation coefficients (CV's) for FGF, TGF- β , IL-1, VEGF, TNF- α , PDGF and Caspase-3 were 5.55%, 5.34%, 4.49%, 5.15%, 5.10%, 5.28%, and 5.42%, respectively. The reported interassay CV's for FGF, TGF- β , IL-1, VEGF, TNF- α , PDGF and Caspase-3 were 5.27%, 4.79%, 4.90%, 4.93%, 4.76%, 5.44% and 4.84%, respectively.

Statistical analysis

All statistical analysis was performed using SPSS version 20.0 (SPSS; Chicago, IL, USA). The normally distributed data are presented as mean $\pm$ standard deviation (SD) and non-normally distributed data are expressed as median (25%–75%). For continuous data One way ANOVA and Bonferroni adjustment tests were used for comparing normally distributed data. Kruskal-Wallis test was used for comparing non-normally distributed data. To protect Type 1 error, we used Bonferroni Correction for New alpha values. On The other hand, for controlling and comparison

the data, we used rank transformation to cover the data nonnormally to normaly distributed form and then we performed ANOVA and classical post hoc tests. A p value of <0.05 was accepted as statistically significant.

Results

Macroscopic evaluation: Macroscopic scoring of adhesions was done blind to the study groups by a pathologist. Severe adhesions were observed around the amniotic membrane in the AMT group. In addition, severe adherence was observed in the control group. However, there was no adhesion in the MSC group.

Histopathological evaluation

Fibrosis/adhesion severity and collagen deposit density were evaluated by H/E and MST staining, which were significantly increased in the AMT and the control group, but not increased in the MSC group (Figures 1, 2, Table 1). The tissue repair response after MSC treatment was statistically similar with the untreated group in terms of collagen density and fibrosis ($p > 0.05$, $p = 0.392$). After MSC treatment, the tissue repair response was statistically similar with the control group in terms of histiosis, vascularisation, granulocytic response ($p > 0.05$, $p > 0.05$, $p > 0.05$). However, the tissue repair response after AMT treatment was statistically similar with the control group in terms of collagen density and fibrosis ($p > 0.05$, $p > 0.05$). At low power magnification, the distribution of inflammatory cell infiltration was relatively in homogeneous within slides. Lymphocytes infiltrated tissue in lymphoid aggregates or in diffuse manner, and dense lymphocytic infiltrates were detected in stromal areas. Two independent slides that were selected from each rat had a good level of homogeneity for inflammatory cell density, and all cases were classified using the four-degree scale as described above.

Pericardial thickness was graded and compared for all of the H/E, MST, and IF stains. The most pronounced thickening was observed in the AMT group and less thickening was present in the control group, with no pericardial thickening in the MSC group (Table 2: Pericardial thickening (micrometer) and statistical comparison of the groups). There was no statistically significant difference in pericardial thickness between MSC Group and sham group ($p > 0.05$). Fibrous adhesions were observed between parietal and visceral pericardium. These adhesions consisted

Figure 1: Histopathological evaluation of the tissues by Hematoxylin/Eosin staining; Arrows show adhesion/fibrosis of pericardial tissue. MSC: Rat adipose tissue originated Mesenchymal stem cells group. AMT: Rat amniotic membrane transfer group. Group-C (CON): Untreated group. Sham: Not operated group.

of a thick layer of dense connective tissue filled with fibroblastic cells.

Immunofluorescent staining (IFS): The presence of GFP-marked MSC in the area of the cardiac-pericardium was confirmed in all rats in the MSC group (Figure 3).

Biochemical inflammatory parameters

In the evaluation of inflammatory parameters there was no difference between the groups for TGF- β 1 and TNF- α . Other inflammatory parameters showed a significant difference between the groups. All inflammatory markers were found to be significantly higher in the control group (Table 3: Comparison of inflammatory markers).

FGF/VEGF were similar in AMT and MSC groups but lower in the Group-C ($p=0.003$, $p=0.002$, $p=0.009$, $p=0.01$).

PDGF was found to be significantly higher in the Group-C than MSC group, but similar in the Group-C and AMT groups ($p=0.013$, $p=0.261$).

IL-1 was significantly higher in the control group than the AMT group, but similar in control and MSC groups ($p=0.26$, $p=0.182$).

Caspase-3 was found to be significantly higher in the AMT group than the MSC group, ($p=0.04$). This was the only difference between the minimum and maximum successful groups (AMT and MSC) groups (9.82 ± 2.11 ng/mL vs. 6.13 ± 2.63 ng/mL).

During the follow-up period, none of the animals in the groups had any clinical symptoms as a side effect associated with treatments. No animal died during the present study.

No wound infections or dehiscence developed in the area of sternotomy and pericardiotomy.

Discussion

The present study demonstrates that RaMSC application in rats reduces pericardial adhesions, pericardial

Figure 2: Histopathological evaluation of the tissues by Masson's trichrome staining; Arrows show adhesion/fibrosis of pericardial tissue MSC: Rat adipose tissue originated Mesenchymal stem cells group. AMT: Rat amniotic membrane transfer group. Group-C (CON): Untreated group. Sham: Not operated group.

Table 1: Tissue repair response and statistical evaluation

Tissue repair response and statistical evaluation	Sham-MSC	Sham-Group-C	Sham-AMT	MSC- Group-C	MSC-AMT	Group-C-AMT
Collagen density ^a	p > 0.05	p = 0.01	p = 0.007	p = 0.001	p < 0.001	p > 0.05
Fibrosis	p = 0.392	p = 0.009	p = 0.048	p < 0.001	p < 0.001	p > 0.05
Histiosis	p = 0.031	p = 0.007	p = 0.005	p > 0.05	p < 0.001	p = 0.002
Vascularisation	p = 0.011	p = 0.003	p = 0.005	p > 0.05	p < 0.001	p = 0.001
Granulocytic response	p = 0.003	p = 0.005	p = 0.005	p > 0.05	p < 0.001	p = 0.001

^aAll histopathological inflammation parameters evaluated with H/E staining, except collagen density. Collagen Density was evaluated with Masson's Trichrome staining. Bold figures indicate statistical significance.

Table 2: Statistical comparison of pericardial thickness among the groups.

Differences among the groups	Sham- Group-C	Sham-AMT	Sham-MSC	Group-C-AMT	Group-C -MSC	AMT-MSC
Pericardial thickness	p < 0.001	p = 0.014	p < 0.001	p > 0.05	p < 0.001	p < 0.001

MSC: Rat adipose tissue originated Mesenchymal stem cells applied group. AMT: Rat amniotic membrane transfer applied group. Group-C: Not treated group. Sham: Not operated group. Because of the statistically distribution of pericardial thickness was non-normal Kruskal Wallis Test was performed and the data was expressed median (25%–75%). Bold figures indicate statistical significance.

Figure 3: Histopathological evaluation of the tissues by immunofluorescent staining; Arrows show adhesion/fibrosis of pericardial tissue MSC: Rat adipose tissue originated Mesenchymal stem cells group. AMT: Rat amniotic membrane transfer group. Group-C (CON): Untreated group. Sham: Not operated group.

Table 3: Comparison of inflammatory markers among the groups.

Markers (mean $\pm$ SD)	Sham	Group-C	AMT	MSC	p-Value
FGF (pg/mL)	214.21 $\pm$ 64.95	424.03 $\pm$ 140.39	224.53 $\pm$ 108.71	167.28 $\pm$ 45.31	$p < 0.001$
TGF- β (pg/mL)	108.02 $\pm$ 54.91	170.91 $\pm$ 100.86	94.61 $\pm$ 35.38	82.61 $\pm$ 39.34	$p = 0.064$
IL-1 (pg/mL)	27.93 $\pm$ 18.31	89.63 $\pm$ 37.12	48.68 $\pm$ 25.07	53.20 $\pm$ 19.52	$p = 0.005$
VEGF (pg/mL)	295.37 $\pm$ 73.63	472.73 $\pm$ 108.18	314.22 $\pm$ 120.43	284.22 $\pm$ 39.90	$p = 0.001$
TNF- α (pg/mL)	528.78 $\pm$ 307.03	557.70 $\pm$ 144.77	429.60 $\pm$ 163.18	370.94 $\pm$ 99.61	$p = 0.126$
PDGF (pg/mL)	2.74 $\pm$ 2.21	6.46 $\pm$ 1.71	4.84 $\pm$ 1.88	3.30 $\pm$ 1.32	$p = 0.003$
Caspase-3 (ng/mL)	6.45 $\pm$ 3.42	11.25 $\pm$ 1.95	9.82 $\pm$ 2.11	6.13 $\pm$ 2.63	$p = 0.001$

Because of the data of inflammatory markers have continuous statistical characteristics One Way Anova test was performed and those data were expressed mean $\pm$ SD. MSC: Rat adipose tissue originated Mesenchymal stem cells applied group. AMT: Rat amniotic membrane transfer applied group. Group-C: Not treated group. Sham: Not operated group. Bold figures indicate statistical significance.

thickness, fibrosis, vascularity, and collagen accumulation. However, it was observed that the same positive contribution could not be achieved with amniotic membrane transfer. The 12-week follow-up period of the study was determined to provide safe mid-term results at sufficient levels [17, 18]. The presence of MSC in pericardial tissue was demonstrated by fluorescence microscopy. As a result of macroscopic appearance and histopathological examination with H/E and Masson's trichrome staining, collagen deposits, fibrosis and lack of adhesion were found statistically and clinically similar in the sham group and MSC groups. This leads us to think MSC use is a more appropriate method to decrease pericardial adhesions. Pericardial thickness-fibrosis and collagen deposition-adhesion severities were found to be correlated in all groups. During pericardial repair, fibrin filaments combine visceral and parietal layers without the mesothelial layer. During the healing of the two pericardial layers, if the mesothelial cell layer is also regenerated and the pericardial fibrinolytic effect occurs adequately, the fibrin breaks down and adhesion can be

limited [19]. Matsui et al. investigated the effect of stem cell application on some transcription factors such as STAT-3 and matrix metalloproteinases (MMP) associated with renal fibrotic injury in rats. The study showed that MSCs protect against obstruction-induced renal fibrosis by down regulating STAT-3 activation and STAT-3-induced MMP-9 expression in addition dramatically reducing collagen and fibronectin accumulation [20]. Meier et al. reported that MSC derived soluble molecules form MMP-mediated anti-inflammatory and antifibrotic effects in experimental liver fibrosis [21]. The relationship between inflammation, fibrosis, and adhesion is not yet clearly understood. Nonetheless, it is understood that inflammatory cells mediate fibrosis formation to at least a degree in all examined tissues. In the wound healing process, the presence of some fibroblast-stimulating profibrotics, especially TGF- β , was suggested [22]. The epithelial cells in damaged tissues can produce the majority of profibrotic cytokines such as TGF- β and PDGF [23]. Shah et al. showed that fibrosis and collagen density can be reduced by lowering TGF- β 1 levels. It was also reported that the

use of TGF- β 3 is promising as an anti-scarring agent. But, the exact mechanism by which TGF- β 3 reduces scarring remains to be elucidated. As an inverse approach, it was reported that if the leukocyte pathways are blocked by knockout of some genes, wound healing is possible without fibrosis and scar tissue. Also, when the number of monocytes were decreased by gene knockout, it was observed that TGF- β levels in granulation tissue decreased and because of this fibronectin production and fibrotic repair decreased. According to these results, it is thought that the fibrotic response may be directly related to leukocyte signaling [24]. Anti-inflammatory drugs, even non-steroidal, especially COX-2 inhibitors, were shown to help prevent the development of liver fibrosis in animal models [25]. In accordance with these references, it is not surprising that the adhesion of pericardial layers was limited by the application of RaMSC. Inflammatory and fibrosis-reducing effects of MSC in tissues are well known [9]. Prockop et al. reported that more successful remodeling process was achieved on wound healing by using MSC and that fibrosis can be limited by the regulation of the inflammatory response [26]. Ortiz et al. reported that MSC application significantly reduced inflammation and collagen accumulation, and also protected lung tissue from damage in an study in which pulmonary fibrosis was modeled using bleomycin [27]. Similarly, in a study of rotator cuff damage in rats, it was shown that muscle fibrosis could be reduced by MSC application [28].

In our study, adhesion and fibrosis in the Group-C were more pronounced as expected. Macroscopic and histological results in all animals of the control group showed consistent thickening of the pericardium and thick adhesions. However, it is an unexpected result in our findings that the severity of adhesions around the amnion membrane is even more apparent than in the untreated group [29]. Recently, Khalpey et al. showed that treatment with amniotic membrane and stem cells containing extracellular matrix can reduce fibrosis and post-operative inflammation [30]. Although this advantageous tissue transfer, which does not require immunosuppression therapy and donor compliance, offers positive contributions in some studies, it is not clear why it caused the worst results in our study. Peng et al. used amniotic membranes to prevent intrauterine adhesion in the human body. In this study, hysteroscopy was performed for the follow-up monthly, and at the end of the second month, it was reported that the amniotic membrane had not yet disappeared and positive contribution could not be determined. However, fibrosis was significantly reduced and the transferred membrane became in situ in the third control [18]. Our follow-up period of 12 weeks is compatible with these referenced

studies. In the same study, this was related to the 12-week duration of the wound maturation process. In addition, amniotic membranes are not only a barrier, but also have properties that produce healing and release active substances that affect tissue regeneration. With these properties of the amniotic membrane, it was reported that firstly, fibrosis developed prominently and then began to disappear in the study [31]. In a study report, even after several surgical treatments, it was emphasized that AMT can prevent inflammation and scar formation by comparing a physical barrier between reconstruction materials and brain tissue [12]. Karaca et al. investigated the effect of locally applied mesenchymal stem cells on intra-abdominal adhesions. They showed that topical MSC application immediately after surgery suppresses the inflammatory process [32]. Aydin et al. investigated the effectiveness of platelet-rich plasma and mesenchymal stem cells in wound healing suppressed by corticosteroid in rats. They revealed that platelet-rich plasma could improve the histopathological grades in wound healing which was suppressed by corticosteroid in rats, while mesenchymal stem cells could show their therapeutic effects via biochemical route [33]. When these studies are examined together, it can be observed clearly that these treatments do not block the formation of fibrosis primarily, but rather dissolve fibrosis and adhesions that develop during the maturation of the wound. In the remodeling process, various collagenase enzymes ensure collagen degradation. The fibrous bundles are thickened by combining or degradation. The severity of the scarring and adhesion tissue is determined by apoptosis gradually decreasing in vascularity and the number of inflammatory cells such as fibroblasts and macrophages. It is noted that a very slow-moving remodeling period can continue for up to 2 years; however, it should be considered that this process may change for each tissue or species [34]. This leads us to think that the failure of the AMT method was due to the lack of duration or differences in tissue response. As previously mentioned in the literature, it was thought that fibrosis could regress with a longer follow-up period and adhesions could be reduced.

In our study, high levels of inflammatory markers in all groups, TNF- α , TGF- β , IL-1, PDGF, FGF, VEGF and Caspase-3 [24] may be associated with the continued remodeling process [18]. All inflammatory parameters among the groups were higher in the Group-C. This result was related to the fact that the healing and maturation process in the group was continuing and the severity of inflammation was not limited. The only significant parameter between the MSC and AMT groups was mediator Caspase-3 levels, which is a marker responsible for apoptosis [35].

Conclusion

Findings obtained in our study show that RaMSC administration can achieve a reduction in fibrosis/adhesion, but amniotic membrane transfer did not contribute to reducing adhesions. The significant elevation of Caspase-3 levels in the AMT group suggests a positive correlation between this marker and the postoperative adhesion/fibrosis, so this relationship should be examined in more detail. It will also be useful to investigate whether RaMSC inhibits postoperative peritoneal or pleural adhesions. Finally, it is thought that RaMSC method may help to develop a novel therapy and preventive treatment for postoperative pericardial adhesions in humans.

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