Effectiveness of Dimethylsulfoxide on the Survival and Volume Preservation of Autologous Fat Graft Tissue: A Preliminary Study

Elif Sari, MD; Bulent Bakar, MD; Bahram Sarkarati, MD, PhD; Onder Bozdogan, MD; and Tarik Cavusoglu, MD

Abstract
Background: The survival of autologous fat graft tissue is dependent on various factors, such as vascularization and inflammation.
Objective: This study aims to evaluate the possible beneficial effects of dimethylsulfoxide (DMSO) on fat graft volume and survival.
Methods: Eighteen male Wistar albino rats were divided randomly into three groups. An autologous fat graft obtained from the inguinal region of each rat was transferred to its back. DMSO was administered intraperitoneally (IP) in the DMSO-IP group and cutaneously (C) in the DMSO-C group once daily for 15 days after the surgical procedure. The control group underwent surgery but was not administered with DMSO. Two months after surgery, the grafted fatty tissues were harvested for histopathological and biochemical analyses.
Results: The results showed that 2 months postoperatively, fat grafts of the DMSO-C and DMSO-IP groups weighed significantly more than the grafts of the control group. Moreover, the vascularity of the grafts was higher in the DMSO-C group than in the control group, and no significant difference was found between the two DMSO groups. The mean lipid peroxidation levels were the same in the three groups, but myeloperoxidation was significantly lower in the DMSO-C group than in the other two groups.
Conclusions: The study results showed that cutaneous rather than intraperitoneal DMSO administration could preserve the quality and volume of transplanted fat tissue in rats by enhancing vascularity and decreasing inflammation.

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Autologous fat grafting is a frequently performed procedure that requires only local anesthesia. It is used not only in facial and body contouring surgeries but also in filling in tissue defects caused by burn scars, veloparyngeal insufficiency, trauma, congenital deformity, and tumor surgery.1,2 However, most of the grafted adipocytes undergo apoptosis in the early postoperative period due to hypoxia and ischemia, which occur in response to the decreased vascular supply around the free-fat graft tissue. In fact, graft loss occurs in 40–60% of the cases.3–6 The resorption of the fat graft, which necessitates repetitive graft applications, unpredictable necrosis, and cyst formation, is among the disadvantages of autologous fat grafting.5,6 Moreover, the viability and volume of these grafts are not predictable, which adds more challenges. Vascular endothelial growth factor,8 antitumor necrosis factor-α,7 platelet rich plasma,9 and platelet-rich fibrin2 have been tested to improve autologous fat graft survival and to preserve the volume of the fat tissue. Among the many anti-inflammatory methods tested thus far are adipocyte concentration to increase the number of delivered

Dr Sari is an Assistant Professor in the Department of Plastic, Reconstructive, and Aesthetic Surgery; Dr Bakar is an Associate Professor in the Department of Neurosurgery; and Dr Bozdogan is a Professor in the Department of Pathology at the Kirikkale University Faculty of Medicine, Kirikkale, Turkey. Dr Sarkarati is an Assistant Professor in the Department of Biochemistry at the Hacettepe University Faculty of Medicine, Ankara, Turkey. Dr Cavusoglu is an Associate Professor in the Plastic, Reconstructive, and Aesthetic Surgery Clinic of Memorial Hospital, Ankara, Turkey.

Corresponding Author:
Dr Elif Sari, Kirikkale University Faculty of Medicine, Department of Plastic, Reconstructive and Aesthetic Surgery, Kirikkale; Kirikkale University Faculty of Medicine, 7th km Yahsihan, 71450 Kirikkale, Turkey. E-mail: drelifsanli@hotmail.com
cells, washing of the graft to decrease the concentrations of inflammatory mediators, and the application of low-pressure and atraumatic grafting techniques. However, the results of these attempts have not been encouraging. DMSO is a well-known anti-inflammatory agent, nonenzymatic antioxidant, and scavenger solvent that penetrates the skin immediately after topical application. It has been shown to greatly improve some skin necrosis due to drug extravasation, arterial thrombosis, and glutamate-induced excitotoxic death. It also increases the proliferation of vascular smooth muscle cells and blood flow in injured tissues. These properties of DMSO suggest its use in procedures involving tissue expanders and breast implants. Specifically, the topical application or systemic administration of DMSO could reduce skin flap necrosis by increasing blood flow to the flap.

In this study, we examined the ability of DMSO to improve the volume and the survival of autologous fat grafts by increasing blood flow to the graft tissue. We hypothesized that DMSO could decrease inflammation and adipocyte death based on its ability to suppress myeloperoxidation and lipid peroxidation cascades.

METHODS

Materials

This study was conducted in accordance with the guidelines for the use of laboratory animals in research set by the Ethical Committee of the Ankara Training and Research Hospital (ethic number: 0015/230) between September 2013 and May 2014.

Eighteen male Wistar albino rats ranging from 16 to 18 weeks old (mean, 17 weeks) and weighting 250 to 300 g (mean, 270 g) were used, and they were selected and numbered by random blind research. The animals were randomly divided into three groups using the restricted randomization method (permuted block randomization); this randomization scheme allows for an equal, pre-specified number of subjects to be assigned to each group.

The DMSO-IP group (n = 6) was administered 0.11 g DMSO once a day as an intraperitoneal injection. The solution was prepared by diluting 0.1 mL of DMSO in 0.9 mL of saline.

The DMSO-C group (n = 6) received 0.1 mL of DMSO diluted in 0.9 mL of saline once a day. The solution was applied to the skin surface covering the transplanted fat graft.

The control group (n = 6) underwent fat transplantation but was not administered the experimental drug in any form.

The DMSO solutions were prepared from anhydrous DMSO (Micro Therapeutics, Irvine, CA). The density of anhydrous liquid DMSO is approximately 1.1 g/mL, its intravenous LD50 in rats is 5.2-8.1 g/kg, and its half-life in rat serum is 60-72 hours.

The animals were anesthetized through the intraperitoneal administration of 40 mg of ketamine HCl/kg (Ketalar; Pfizer, New York, USA) and 5 mg of xylazine HCl/kg (Rompun 2%; Bayer HealthCare, Leverkusen, Germany).

Surgery

The sedated animals were placed in the supine position, and an oblique 3 cm incision was made in the left inguinal area, which was cleaned previously with betadine solution. The inguinal fat tissue was excised, wiped with sterile gauze, and weighed on a precision scale (Shimadzu AX-200, Columbia, MD, USA). Neither local anesthetic nor adrenaline was injected prior to fat dissection. The weight of each inguinal fat graft was recorded. After the removal of the fat grafts, the inguinal incision was sutured with 2/0 silk, and the animals were placed in the prone position. A 1 cm incision was made in the dorsal interscapular area, previously cleaned with betadine solution, and a 1 cm × 1 cm pocket was prepared over the panniculus carnosus. The fat tissues were washed with saline, cut with microscissors into 2-4 mm square pieces, and placed into the pockets.

Two months after the fat grafting surgery, the animals were re-sedated according to the same procedure and euthanized by cardiac air embolization. The fat grafts were then removed from the recipient pockets, dried, and weighed again using the same precision scale. Each harvested fat graft was divided into two equal portions; one was placed in 10% buffered formaldehyde for histopathological examination and the other was immediately stored at −30°C in dry air for biochemical evaluation.

We waited 2 months before removing the grafts because of the inherent difficulty in determining adipocyte viability after it has been fixed in formaldehyde, and because some cells had already undergone apoptosis and still retained much of their original architecture when viewed under normal hematoxylin and eosin (H&E). In this period, non-viable adipocytes would be absorbed or filled with scar tissue.

Histopathology

Tissue samples were fixed in 10% buffered formaldehyde and processed for routine light microscopy observation. Serial sections of 5 μm thickness stained with H&E were examined and photographed. Images were obtained with a DSRII digital camera (Nikon’s inverted Eclipse Ti microscope, equipped with an automatic scanning table; Nikon...
France, Champigny sur Marne, France) connected to the microscope. The area covered by adipocytes was measured at 40× magnification in five areas per graft section using NISElements Advanced Research software version 4.0 (Nikon Instruments, Tokyo, Japan). The average total area (µm²) occupied by adipocytes was expressed as a percentage of the total microscopy viewing area. This ratio is referred to as the relative adipocyte index (Figure 3). The number of blood vessels was counted at 10× magnification in four areas per section using an Olympus BX50 (Olympus Optical Co., Hamburg, Germany) microscope and expressed as the total vessel number (ie, vascularity). Inflammation was measured using an inflammation scale (Table 1). Fat necrosis was evaluated histologically using the same microscope.

Biochemical Analysis

Myeloperoxidation (MPO) and lipid peroxidation (LPO) of the fat graft tissues obtained from the dorsal pockets of the animals were measured spectrophotometrically using a Shimadzu UV-1700 spectrophotometer.30,31 All specimens were evaluated by an experienced biochemist blinded to the study groups and to the sources of the experimental material. LPO was measured spectrophotometrically at 532 nm in thiobarbituric acid–treated samples and expressed as nanomoles per gram of wet tissue. MPO was measured spectrophotometrically at 655 nm in tetramethylbenzidine–treated tissues and expressed as units per gram of wet tissue according to the change in absorbance.30,31

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS/PC 17.0). A normality test was performed in the beginning of the statistical analysis.

As the weights of the fat graft tissues before and after implantation and the LPO levels of the fat graft tissues were normally distributed, and the variations were homogenous between all groups, they were statistically analyzed using a one-way analysis of variance (ANOVA). To determine the statistical differences among the groups, a one-way ANOVA followed by Tukey’s multiple comparison test were performed as a post hoc evaluation. A paired samples t test was used to identify the statistically significant differences between the weights of the fatty tissues from all three groups before and after implantation. Data on tissue MPO levels, vascularity, fat necrosis levels, and inflammation grade were not normally distributed, and the variation was not homogenous among all groups according to the SPSS results (Table 2). Thus, the Kruskal-Wallis multiple variant analysis had to be conducted. The Mann-Whitney U test was used to determine the statistically significant differences (post hoc evaluation) among the groups. Significance was defined at $p < 0.05$. 

Figure 1. Surgical procedures. (A) Fat graft pieces were cut into small pieces before their transplantation onto the back of the rat. The fat graft before its removal from rats in (B) the control group, (C) the dimethylsulfoxide intraperitoneal (DMSO-IP) group, and (D) the dimethylsulfoxide cutaneous (DMSO-C) group. Dense vascularization of the graft tissue was observed in the DMSO-IP and DMSO-C groups.

Figure 2. Schematic illustration of the experimental design. Group 1, control group; Group 2, dimethylsulfoxide intraperitoneal (DMSO-IP) group; Group 3, dimethylsulfoxide cutaneous (DMSO-C) group.
RESULTS

Macroscopic Findings

No local inflammation or abscesses developed in any of the surgical areas. None of the rats died during the 2-month study.

Macroscopic inspection showed that the vascularity of the DMSO-C and DMSO-IP groups were higher than that of the control group. The volumes of the preserved fat tissues were also higher in both DMSO groups than in the control group (Figure 1). During the sample collection period, a fibrous capsule formed around all graft tissues.

Fat Tissue Weights

The weights of the inguinal fat grafts before implantation were not statistically different among the three groups (ANOVA; $F = 2.896, p = 0.086$; Table 3), but at the end of the study, the graft weights differed significantly (ANOVA; $F = 8.040, p = 0.004$). The post hoc analyses showed that the DMSO-C and the DMSO-IP groups differed significantly from the control group (Tukey’s multiple comparison test; $p = 0.013$ and $p = 0.007$, respectively), but not from each other ($p = 0.944$). Specifically, the weights of the implanted and later harvested fat tissues were higher in the DMSO-C and DMSO-IP groups than in the control group. In the control group, the fat tissue weights were significantly higher before than after the grafts were harvested ($t = 2.531, p = 0.022$), but this was not the case in the DMSO-C and DMSO-IP groups (Table 4, Figure 4). Moreover, more fat volume loss was observed in the control group than in the DMSO groups. This observation suggests the preservation of fat graft weight by DMSO, whether applied cutaneously or injected intraperitoneally. Our results also showed that our graft weight loss was lower than that in the literature. We attribute the volume protection to the neovascularization and regenerating zones due to the excision method of the fat tissue.28

Histopathological Findings

Neither the amount of fibrotic tissue around the fat grafts, the inflammation grade, nor the degree of fat necrosis differed among the three groups ($\chi^2 = 3.263, p = 0.196$; $\chi^2 = 1.998, p = 0.368$; and $\chi^2 = 0.442, p = 0.802$, respectively; Tables 5 and 6). By contrast, significant differences were found in vascularity (ANOVA; $F = 3.876, p = 0.044$).

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**Figure 3.** Histopathologically based relative adipocyte index in the three groups. (A) Control group, (B) dimethylsulfoxide intraperitoneal (DMSO-IP) group, and (C) dimethylsulfoxide cutaneous (DMSO-C) group.

**Table 1.** Histopathological Inflammation Scale Used to Determine Inflammation Levels in the Dimethylsulfoxide and Control Groups

<table>
<thead>
<tr>
<th>Grade Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>No inflammation or scattered/few lymphocytes</td>
</tr>
<tr>
<td>Grade 1</td>
<td>A small group of lymphocytes (10-20)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>A large group of lymphocytes (&gt;20), or two small groups</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Diffuse lymphocyte infiltration or more than one large group of lymphocytes</td>
</tr>
</tbody>
</table>

**Table 2.** Normality Test Results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Kolmogorov-Smirnov</th>
<th>Shapiro-Wilk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>Sig.</td>
</tr>
<tr>
<td>Weight before</td>
<td>18</td>
<td>0.20</td>
</tr>
<tr>
<td>Weight after</td>
<td>18</td>
<td>0.20</td>
</tr>
<tr>
<td>Total area</td>
<td>18</td>
<td>0.00</td>
</tr>
<tr>
<td>Fibrotic area</td>
<td>18</td>
<td>0.00</td>
</tr>
<tr>
<td>LPO</td>
<td>18</td>
<td>0.20</td>
</tr>
<tr>
<td>MPO</td>
<td>18</td>
<td>0.00</td>
</tr>
</tbody>
</table>

LPO, lipid peroxidation; MPO, myeloperoxidation.
The post hoc evaluation showed significantly higher vascularity in the DMSO-C group than in the control group (Tukey’s multiple comparison test; \( p = 0.044 \)), and no significant differences were found between the control and the DMSO-IP groups or between the two DMSO groups (\( p = 0.136 \) and \( p = 0.841 \), respectively; Figures 5 and 6).

LPO and MPO

No differences were found in the mean LPO levels of the three groups (\( F = 0.082, p = 0.921 \); Figure 7), and the differences in the mean MPO levels of the control, DMSO-C, and DMSO-IP groups were significant (\( \chi^2 = 11.524, p = 0.003 \); Table 6). The post hoc evaluation showed that the MPO levels were significantly lower in the DMSO-C group than in the control and in the DMSO-C group versus the DMSO-IP group (\( Z = -2.882, p = 0.004 \) and \( Z = -2.882, p = 0.004 \), respectively; Figure 7). Thus, DMSO appears to have decreased the inflammatory cytokine levels, although this finding remains to be confirmed in a more detailed biochemical analysis.

DISCUSSION

DMSO can cross most biological membranes and the skin quickly and easily because it is highly liposoluble and water soluble. It has also been used to provide pain relief based on its ability to block peripheral nerve C-fibers. Recently, cutaneously applied and/or parenterally administered DMSO has been used postoperatively to improve skin flap survival in plastic surgery because it increases flap perfusion.\(^{17,18,27,32,33}\)

In this study, the ability of DMSO to preserve fat graft volumes and viability was examined together with its anti-inflammatory effects and its positive effects on vascularization. The efficacy of cutaneously applied versus intraperitoneally injected DMSO was determined. Our results showed that in terms of preserving fat graft volumes, the two methods were equally effective. However, histopathological analyses showed that neither route decreased or blocked the development of fibrotic tissue and fat tissue necrosis. Nonetheless, vascularity in the DMSO-C group was higher than that in the control group values, but was not significantly different from the DMSO-IP group. Despite the absence of a statistically significant difference between the control and the DMSO-IP group, the mean vascularity in the latter tended to be higher. Therefore, DMSO seems to increase the vascularization of fat graft tissue.

In many studies, fat graft loss is caused by the development of hypoxic and ischemic conditions because of the decreased vascular supply around the free-fat graft tissue.\(^{3-6}\) Hypoxia generates potent oxidizing species (eg, superoxide radical, hydroxyl radical, and hydrogen peroxide), which

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**Table 3. The Fat Graft Weights in the Dimethylsulfoxide and Control Groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Before</td>
<td>0.27</td>
<td>0.72</td>
<td>0.45</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.12</td>
<td>0.35</td>
<td>0.20</td>
<td>0.09</td>
</tr>
<tr>
<td>DMSO-C</td>
<td>Before</td>
<td>0.52</td>
<td>0.87</td>
<td>0.64</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.28</td>
<td>0.74</td>
<td>0.52</td>
<td>0.20</td>
</tr>
<tr>
<td>DMSO-IP</td>
<td>Before</td>
<td>0.42</td>
<td>0.69</td>
<td>0.58</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.25</td>
<td>0.69</td>
<td>0.49</td>
<td>0.17</td>
</tr>
</tbody>
</table>

DMSO-C, dimethylsulfoxide cutaneous; DMSO-IP, dimethylsulfoxide intraperitoneal; SD, standard deviation.

**Table 4. Comparisons of the Fat Graft Weights Before and After Implantation According to a Paired Samples \( t \)-Test**

<table>
<thead>
<tr>
<th>Group</th>
<th>( t )</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.531</td>
<td>0.022</td>
</tr>
<tr>
<td>DMSO-IP</td>
<td>0.873</td>
<td>0.423</td>
</tr>
<tr>
<td>DMSO-C</td>
<td>0.973</td>
<td>0.375</td>
</tr>
</tbody>
</table>

\( p < 0.05 \) Bold values indicates \( p < 0.05 \). The \( p \) values which are lower than 0.05 are significant.

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**Figure 4.** Weights of the fat graft of the DMSO groups versus the control group. Error bars show the minimum and the maximum weights of the fat grafts. More fat graft weight loss could be seen in the control group than in the DMSO groups. Dimethylsulfoxide cutaneous (DMSO-C) administration is more effective than dimethylsulfoxide intraperitoneal (DMSO-IP) delivery in terms of weight preservation.
mediate the lethal cell injury by initiating the peroxidative decomposition of phospholipids, both in the cellular and mitochondrial membranes. MPO activity was previously shown to correlate with the absolute number of phagocytes and their activation in the inflamed tissue. Neutrophils and other phagocytes (e.g., macrophages) produce hypochloride, a strong oxidant that acts on hydrogen peroxide and chloride ion during MPO. LPO plays an important role in the pathogenesis of lethal cell injury by degrading cellular and mitochondrial membranes. In our study, the MPO levels were lower in the DMSO-C group than in the control and DMSO-IP groups, and no differences were found in the amount of inflammation or in LPO levels. Therefore, although topical DMSO seemed to decrease the production of cytotoxic agents at the graft site, it could not inhibit the LPO cascades. The reason why neither the topically applied nor the intraperitoneally injected DMSO exhibited its well-established antioxidant and free radical scavenger properties in the transplanted fat grafts in the rats is unclear. These results could be interpreted as apoptosis, but our study did not contain apoptotic pathway investigation results because of some financial limitations. Instead, DMSO protected or even increased the fat volume through neovascularization, and supported fat cell regeneration by protecting the surviving and regenerating zones of the graft. Eto et al showed that proliferation began from the regenerating zone of the grafted fat tissue on the third day, consistent with our

### Table 5. Tissue Levels in the Control, DMSO-IP, and DMSO-C Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Fibrosis</td>
<td>240,144</td>
<td>1,365,592</td>
<td>588489.83</td>
<td>446611.56</td>
</tr>
<tr>
<td></td>
<td>Inflammation</td>
<td>0.00</td>
<td>3.00</td>
<td>1.17</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Fat necrosis</td>
<td>0.00</td>
<td>1.00</td>
<td>0.33</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>Vascularity</td>
<td>17.40</td>
<td>33.60</td>
<td>27.43</td>
<td>6.25</td>
</tr>
<tr>
<td></td>
<td>LPO</td>
<td>29.69</td>
<td>58.85</td>
<td>46.23</td>
<td>11.70</td>
</tr>
<tr>
<td></td>
<td>MPO</td>
<td>7.20</td>
<td>43.80</td>
<td>19.14</td>
<td>12.84</td>
</tr>
<tr>
<td>DMSO-C</td>
<td>Fibrosis</td>
<td>20,501</td>
<td>864,834</td>
<td>336776.33</td>
<td>296146.46</td>
</tr>
<tr>
<td></td>
<td>Inflammation</td>
<td>0.00</td>
<td>3.00</td>
<td>1.83</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Fat necrosis</td>
<td>0.00</td>
<td>1.00</td>
<td>0.50</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Vascularity</td>
<td>31.40</td>
<td>51.40</td>
<td>39.21</td>
<td>6.92</td>
</tr>
<tr>
<td></td>
<td>LPO</td>
<td>16.54</td>
<td>94.27</td>
<td>50.59</td>
<td>26.29</td>
</tr>
<tr>
<td></td>
<td>MPO</td>
<td>0.20</td>
<td>6.50</td>
<td>2.87</td>
<td>2.61</td>
</tr>
<tr>
<td>DMSO-IP</td>
<td>Fibrosis</td>
<td>355,415</td>
<td>1,225,042</td>
<td>551707.33</td>
<td>332491.72</td>
</tr>
<tr>
<td></td>
<td>Inflammation</td>
<td>0.00</td>
<td>3.00</td>
<td>1.33</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>Fat necrosis</td>
<td>0.00</td>
<td>1.00</td>
<td>0.33</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>Vascularity</td>
<td>23.50</td>
<td>49.00</td>
<td>36.433</td>
<td>9.46</td>
</tr>
<tr>
<td></td>
<td>LPO</td>
<td>32.69</td>
<td>84.62</td>
<td>49.81</td>
<td>18.74</td>
</tr>
<tr>
<td></td>
<td>MPO</td>
<td>7.20</td>
<td>99.50</td>
<td>42.93</td>
<td>43.24</td>
</tr>
</tbody>
</table>

DMSO-C, dimethylsulfoxide cutaneous; DMSO-IP, dimethylsulfoxide intraperitoneal; LPO, lipid peroxidation; MPO, myeloperoxidation; SD, standard deviation. Fat necrosis results are in area, µm²; fibrosis, area, µm²; inflammation, score; lipid peroxidation, nmol/g protein; myeloperoxidation, U/g protein; vascularity, five areas per section.

### Table 6. Comparison of Myeloperoxidation in the Control, DMSO-IP, and DMSO-C Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>X²</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis</td>
<td>3.263</td>
<td>2</td>
<td>0.196</td>
</tr>
<tr>
<td>Inflammation</td>
<td>1.998</td>
<td>2</td>
<td>0.368</td>
</tr>
<tr>
<td>Fat necrosis</td>
<td>0.442</td>
<td>2</td>
<td>0.802</td>
</tr>
<tr>
<td>MPO</td>
<td>11.524</td>
<td>2</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

Using the Kruskal-Wallis Multiple Variant Analysis. DMSO-C, dimethylsulfoxide cutaneous; DMSO-IP, dimethylsulfoxide intraperitoneal; MPO, myeloperoxidation. *Bold value indicates p < 0.05. The p values which are lower than 0.05 are significant.
own findings. The inability of intraperitoneally injected DMSO to reduce or block the MPO cascades could be due to the rapid dissolution of DMSO in the blood and its rapid transport away from the vascular circulation to the extravascular compartments. These events would have restricted the accumulation of DMSO in the fat graft tissue and, therefore, its scavenger effects. By contrast, cutaneously applied DMSO would have been concentrated at the graft site.

In the present study, DMSO was not used with local anesthetic solutions, as recent studies have shown that local anesthetic solutions do not affect the fat graft survival. Moreover, in several studies in the literature, the fat grafts were washed with saline to eliminate the effect of Klein solution after harvesting. Thus, we aimed to investigate the individual effects of DMSO on fat graft survival in our study. Nevertheless, future studies should be conducted using the Klein solution, of course.

The experimental method of this article was constructed according to the literature. Thus, we aimed to use the benefits of the anti-inflammatory and vascularization-enhancing effects of DMSO in first 2 weeks after the transplantation. We could not perform an immunohistochemical analysis (eg, perilipin staining) because of financial limitations. Thus, the dying adipocytes could not be detected by

Figure 5. (A) Inflammation, (B) vascularity, (C) fat necrosis, and (D) fibrosis in the dimethylsulfoxide (DMSO) groups versus the control group. Error bars show the minimum and the maximum vascularity, fibrotic area diameter, degree of inflammation, and fat necrosis. Although DMSO did not decrease the fat graft necrosis and inflammatory responses, it did protect the fat graft volume. Moreover, systemic and topical DMSO could indirectly improve fat graft survival by increasing the vascularity of autologous fat graft tissue. DMSO-C, dimethylsulfoxide cutaneous administration group; DMSO-IP, dimethylsulfoxide intraperitoneal administration group.
H&E in the early stages of the study. We decided to wait for 2 months for the results, as the non-viable adipocytes would be absorbed or filled with scar tissue in the long-term. We observed a fibrotic capsule around the transplanted fat tissue at the end of 2 months. According to Eto et al, greater amounts of adipocytes are ruptured in aspirated adipose tissue than in excised tissue. Stromal cells are higher in excised adipose tissue than in aspirated tissue. Perhaps excision could be responsible for the fibrotic capsule that facilitated the fat removal.

Clinical studies demonstrated that high-dose intravenous DMSO produces various adverse effects, such as nausea, vomiting, flushing, fever, chills, dyspnea, cardiac symptoms, transient hypertension, hypotension, anaphylaxis, encephalopathy, amnesia, and seizures, whereas the side effects of long-term cutaneous application of DMSO at concentrations below 50% are negligible. Cutaneous applications of DMSO in humans for scleroderma, ischemic ulcers, decubitus ulcers, and skin necrosis due to antineoplastic agent extravasation have been mentioned in the literature. Variable concentrations of DMSO gels and creams were approved by the Food and Drug Administration in the 1970s. These formulas may be useful for further human studies. Therefore, for human use, daily topical application of 50% DMSO solutions over the fat grafted area may offer a quick, simple, and painless method for graft protection that can be easily applied by the patient alone. Several molecules have been described to enhance fat graft survival in the literature. Conversely, the present study adds a new and effective drug to the relevant literature that can be practically applied over the skin.

Our study has some limitations. First, more sophisticated biochemical analyses used to dissect the inflammatory pathways that influence fat graft survival and volume.

Figure 6. Histopathological illustration of the three groups. (A) Control, (B) dimethylsulfoxide cutaneous (DMSO-C), and (C) dimethylsulfoxide intraperitoneal (DMSO-IP).

Figure 7. (A) Lipid peroxidation (LPO) and (B) myeloperoxidation (MPO) in the DMSO groups versus the control group. Error bars show the minimum and the maximum levels of LPO and MPO. The MPO levels were lower in the dimethylsulfoxide cutaneous (DMSO-C) group than in the control and dimethylsulfoxide intraperitoneal (DMSO-IP) groups, and no differences were found in the amount of inflammation or in the LPO levels.
apoptosis, glutathione and nitrite/nitrate levels, and oxidase activity) were not possible in our laboratory. Thus, the mechanism of the effect of DMSO on LPO and MPO cascades remains to be determined. Second, although a G-power analysis was conducted for the groups, the ethical committee did not allow us to use larger groups of rats. Therefore, future studies could use study groups with a larger size. Third, excised and aspirated adipose tissues have different characteristics in clinical practice. Therefore, aspirated fat tissue with vasoconstrictor solutions can be evaluated in future studies. Fourth, non-vascularized fat grafts obtain their nutrients through plasmatic diffusion from surrounding tissues until neovascularization begins. Therefore, peripherally located adipocytes can much better withstand ischemic conditions. Theoretically, fat graft survivability could be better in well-vascularized tissues than in poorly vascularized tissues. However, we believe that the human body has a biological compensatory system to salvage tissues on its own. Nevertheless, we aimed to study the effectiveness of DMSO on fat graft volume in the health rat model. In accordance with this aim, we did not study traumatized recipient sites (eg, radiated breast tissue). To explain the variability in fat graft survivability between well-vascularized and poorly-vascularized areas, DMSO could be used in these different regions in future studies. Fifth, as the fat tissues harvested from each rat were in different volumes, we could not standardize the weights before the transfer. Thus, the results were compared with the beginning values. Otherwise, we were forced to delay the surgical procedures of other animals.

CONCLUSION

Our study showed that although DMSO did not decrease the fat graft necrosis and inflammatory responses, it did protect the fat graft volume. Moreover, systemic and topical DMSO may indirectly improve fat graft survival by increasing the vascularity of autologous fat graft tissue. The topical application of DMSO seems to be much more effective than intraperitoneal administration and offers a safe, simple, quick, and painless method of use.

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