Tropical Journal of Pharmaceutical Research February 2023; 22 (2): 343-348
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i2.17

Original Research Article

Effect of adipose-derived stem cells on the survival rate of long random pattern skin flap in rats, and the underlying mechanism of action

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Sent for review: 28 October 2022 *Revised accepted: 26 January 2023*

Abstract

Purpose: *To investigate the effect of adipose-derived stem cells (ADSCs) on the survival rate of long random pattern skin flaps in rats and the likely underlying mechanism.*
Methods: *Twenty (20) SPF-grade SD rats were randomly divided into control group and study group, with 10 rats in each group. Rats in the control group were given phosphate buffered saline (PBS), while rats in the study group received ADSCs. The survival rate of the skin flaps was compared between the 2 groups on day 7 after operation, while the levels of indices related to oxidative stress and inflammatory response were compared on the 7th and 14th days after operation.*
Results: *Necrosis in the study group was milder ($p < 0.05$). On the 7th post-operation day, the survival rate of rat skin flap in the study group was significantly higher than that in the control group ($p < 0.05$), but the MDA level in the study group was lower than that of the control group ($p < 0.05$). On the 14th day after the operation, the MDA level was decreased in the two groups ($p < 0.05$), with a lower MDA level in the study group than in the control group ($p < 0.05$).*
Conclusion: *ADSCs inhibit inflammatory response and reduces the level of oxidative stress after long random pattern skin flap transplantation in rats, thereby enhancing blood supply to the flap transplantation area and improving its survival rate. Thus, ADSCs have a potential clinical application in enhancing blood supply in skin transplantation.*

Keywords: *Adipose-derived stem cells, Long random pattern skin flap, Survival rate, Oxidative stress, Inflammatory response*

INTRODUCTION

The skin is the largest tissue of the human body, and it protects the organs and tissues and maintains metabolism. Skin flap transplantation is often used for the clinical treatment of large-scale skin defects such as trauma, infection, surgical resection, exposed organs, and deformity, all of which are difficult to repair by skin grafting [1,2].

The survival rate of the flap after transplantation is closely related to the source and area of the skin material, blood supply, and immune compatibility. When the length-to-width ratio of the grafted skin flap is too large, avascular necrosis at the distal end of the skin flap often occurs [3]. In recent years, stem cell transplantation has become one of the research focuses of wound repair and regeneration. Adipose-derived stem cells (ADSCs) were first reported 21 years ago [4]. Studies have shown that ADSCs are similar to bone marrow-derived mesenchymal stem cells, with respect to low immunogenicity, multi-directional differentiation potential, and potential for self-replication and renewal. Moreover, ADSCs can differentiate into mesoderm chondrocytes, myoblasts or osteoblasts, suggesting their potential benefits in wound repair. A unit volume of adipose tissue produces more mesenchymal stem cells than umbilical cord blood and bone marrow [5].

This study was aimed at investigating the effect of ADSCs on the survival rate of long random pattern skin flap (LRPSF) in rats, and the underlying mechanism.

EXPERIMENTAL

Animals

A total of 20 SPF-grade Sprague-Dawley (SD) rats of either gender (mean weight = 250 ± 50 g), aged 2 - 3 months, were randomly divided into a control group and study group using the random number table, with 10 rats in each group. The rats were adaptively fed for 2 weeks before the study, and they were housed in single cages at room temperature of 23 ± 1 °C and 50 % humidity, with free access to water. The rats were fed special diet based on body weight. The animals were kept in an environment with 12-h light/12-h dark photoperiod. Approval for the study was received from the Animal Ethics Committee of the Animal Ethical Committee of Suzhou Municipal Hospital (approval no. SMH2) and the study was conducted in line with the guidelines of "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [6].

Preparation and identification of ADSCs

The adipose tissue used in the experiments was voluntarily donated by patients admitted to the Department of Plastic Surgery of our hospital for liposuction of the waist and abdomen. Sterilized adipose tissue (10 mL) was rinsed with phosphate-buffered saline (PBS) in a centrifuge tube, followed by the addition of 10 mL of 0.2 % type I collagenase (GIBCO, USA). Then, the sample was digested in a 37-°C incubator. Digestion was stopped by adding an equal volume of complete medium to the centrifuge tube, followed by centrifugation at 258 g for 5 min. The supernatant was discarded. The cells in the pelleted complete medium were resuspended, inoculated into culture flasks, and cultured in a 5-% CO_2, 37-°C incubator (Thermo, USA). Passage was conducted when the cells became 80 % confluent. The cell morphology of third-generation ADSCs was examined under a light microscope. The cell surface markers CD34, CD45, CD49d, CD90, CD105, and CD106 were identified using a flow cytometer (Becton, Dickinson, and Company, USA). The antibodies used were purchased from Becton (Dickinson and Company, USA, and Abcam, USA). Adipogenic induction was used to observe the cell surface markers under a light microscope on day 3 and day 14, while identification was done with Oil Red O fat staining method on day 14. The ADSCs appeared as long fusiform adherent growths with swirled distribution under the light microscope. Transparent lipid droplets were formed in the cytoplasm on day 3 of adipogenic induction, and the fusion of lipid droplets was more plump on day 14. Results from flow cytometry showed that CD45, CD34, and CD106 were negatively expressed, while CD49d, CD90, and CD105 were positively expressed. After 14 days, adipogenic induction was identified as positive using Oil Red O fat staining, indicating that ADSCs were successfully prepared. The concentration of ADSCs was adjusted prior to use.

Establishment of a rat model of extra-long random skin flaps

Based on the Mcfarlane flap preparation method [7], the midline of the back of each rat was taken as the sampling center, and the LRPSF was cut. Rats were anesthetized with an intraperitoneal injection of 10 % chloral hydrate at a dose of 100 mg/kg. Under anesthesia, each rat was fixed on the bracket, the back of the rat was fully exposed, and a razor blade was used to depilate the head and neck junction to the sacrococcygeal region, so as to prepare the skin. The LRPSF was designed with the midline of the back of the rat as the center, with a size of 9.0 cm × 3.0 cm, and the flap was divided into 3 areas. From the caudal end to the cephalic end, there were the proximal area, middle area, and distal area, each of size 3 cm × 3 cm. The pedicle of the flap was located about 1.5 cm from the caudal end, and the depth reached the fascia layer. The flap was lifted and sutured *in situ* after complete hemostasis. Care was taken to protect the capillaries and strictly follow aseptic procedures during the peeling of the flap. The wounds were disinfected with iodine twice daily within 7 days after operation. The feeding environment was the same as before surgery. In this study, all models were successfully prepared, and no rats died or fell off.

Treatments

In the study group, 400 μL of ADSC solution equivalent to 1.5 × 10^5 ADSCs was injected at two symmetrical points along the midline in each of the three areas of the flap. For the control group, 400 mL of PBS was injected at similar points along the midline in each of the three areas of the skin flap, in place of ADSC solution. The above treatments were performed once a day for 7 consecutive days.

Evaluation of parameters/indices

General assessment

Necrosis of the skin flap was judged by the color of the flap (black), weakening and hardening of elasticity, and decrease in skin temperature. The color and elasticity, skin temperature, and edema of the flaps in the proximal, middle, and distal regions were examined and recorded daily, and photographs were taken on days 3 and 7 after surgery.

Survival rate of skin flap

On the 7th day after the operation, the rats were anesthetized *via* intraperitoneal anesthesia, and the surviving area of the stroked skin flap on the back of the rats was covered with transparent paper [8]. A scale was marked on each photograph taken. The area of the skin flap was counted, and the flap survival rate was calculated as the ratio of viable skin flap to total skin flap, expressed as a percentage, using Image Pro Plus 6.0 image processing software.

Oxidative stress response levels

On the 7th and 14th days after the operation, a 1cm×1cm skin flap was cut from the middle of the skin, and blood was washed off with ice-cold saline. The skin flap was used to prepare a 100 g/L tissue homogenate which was centrifuged at 3500 rad/min at 4 °C for 15 min. Malondialdehyde (MDA) level in the supernatant was measured with thiobarbituric acid chromatometry, while the activity of superoxide dismutase (SOD) was assayed using the pyrogallol auto-oxidation method.

Levels of inflammatory factors

The levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in the above supernatant were measured using enzyme-linked immunosorbent assay (ELISA) kits as per the manufacturer's instructions.

Statistical analysis

Data processing was done with SPSS version 22.0. Measurement data are expressed as mean ± standard deviation (SD). The paired comparison was done with a *t*-test. Values of $p < 0.05$ were taken as indicative of statistically significant differences.

RESULTS

General features of the skin flaps of rats

Rats in both groups survived under anesthesia without infection, effusion, and hematoma. On the 3rd day after the operation, the skin flaps in the distal region of the rats in both groups were blackened and necrotic, with significantly reduced elasticity. There were no obvious changes in the proximal and middle regions, and no obvious swelling and shrinkage occurred. In contrast, there was obvious necrosis of the skin flap of rats in the control group. On the 7th day after the operation, the area of skin flap necrosis was increased in both groups. Moreover, skin flap shrinkage and swelling appeared in both groups. The necrotic zone in the control group reached the middle area. In contrast to the control group, the skin flaps in the proximal and the middle areas of rats in the study group were basically normal.

Survival rate of skin flaps of rats

On the 7th day after the operation, the flap survival rate of the study group was significantly higher than that of the control group ($p < 0.05$; Table 1).

Table 1: Comparison of survival rate of skin flaps between the two groups after 7 days (%, n = 10)

Group	Flap survival rate after surgery (%)
Control	60.76±4.61
Study	78.37±5.08
t	8.118
P-value	0.000

MDA and SOD levels of rats

On the 7th day after the operation, the MDA level of rats in the study group was significantly lower than that of the control group, while the SOD activity was higher than that of the control group. On the 14th day after the operation, there were marked decreases in MDA level of rats in each group. However, MDA level in the study group was significantly lower than that in the control group. At the same time, although SOD activity was markedly increased in the two groups, there was significantly higher SOD activity in the study group than in the control group ($p < 0.05$). These results are shown in Table 2.

Levels of TNF-α and IL-6

On the 7th day after surgery, the levels of TNF-α and IL-6 in the study group were significantly lower than those in the control group. On day 14 day after surgery, although the levels of TNF-α and IL-6 in the two groups were decreased, there were markedly lower levels in the study group than in the control group ($p < 0.05$). These data are shown in Table 3.

DISCUSSION

Some of the major causes of skin tissue damage are usually caused burns, blast injuries, and mechanical injuries. The healing and repair of skin are related to multiple factors such as cell proliferation, oxidative stress, inflammatory response, and extracellular matrix [9,10]. Clinically, skin flap transplantation is normally used as a treatment method for wound repair, recovery of appearance, and functional reconstruction. The establishment of a vascular connection between the skin flap and surrounding tissues after transplantation is one of the key factors that influence the success of skin flap transplantation [11,12]. However, its clinical application is relatively limited due to the excessive length and width of the random flap, insufficient blood supply from well-known blood vessels, and numerous sequelae of postoperative blood supply disorders. When the ischemia time is too long, necrosis easily occurs. On the restoration of blood supply, there is a release of large amounts of inflammatory mediators and reactive oxygen species, resulting in pathophysiological changes such as cell edema and apoptosis which aggravate tissue damage and cause distal necrosis of the skin flap [13,14].

Angiogenesis is comprised of multiple complex regulatory processes such as endothelial cell proliferation, activation, migration, invasion, germination, basement membrane formation, and new blood vessel maturation. Studies have confirmed that stem cell therapy plays a key role in promoting the formation of new blood vessels. In recent years, stem cell transplantation therapy has become an important area of focus in wound repair. Research on embryonic stem cells and bone marrow mesenchymal stem cells have become more in-depth, although their clinical applications are limited due to immune rejection, difficulty in obtaining materials, and legal constraints [15].

Adipose-derived stem cells (ADSCs) are mesenchymal stem cells in adipose tissue which have multiple differentiation potential: they can be induced to differentiate into cells derived from various germ layers.

Table 2: Comparison of MDA and SOD levels of rats between the two groups at 7 and 14 days post-surgery (n = 10)

Group	MDA (nmol/mg)		SOD (U/g)	
	7 days after operation	***14 days after operation***	***7 days after operation***	***14 days after operation***
Control	9.72±1.39	7.12±0.98*	46.73±7.53	58.23±8.59*
Study	4.06±0.42	3.28±0.52*	122.06±10.13	143.91±13.26*
t	12.326	10.946	18.873	17.149
P-value	0.000	0.000	0.000	0.000

*$P < 0.05$, compared with the same group before treatment

Table 3: Comparison of the levels of TNF-α and IL-6 between the two groups at 7 and 14 days post-surgery (pg/mL)

Group	TNF-α		IL-6	
	7 days after operation	***14 days after operation***	***7 days after operation***	***14 days after operation***
Control	2592.07±217.39	1683.10±160.39*	2216.53±202.75	1265.22±143.25*
Study	1580.96±172.43	1213.62±141.52*	1861.01±193.70	943.69±131.72*
t	11.523	6.941	4.009	5.225
P-value	0.000	0.000	0.001	0.000

*$P < 0.05$, compared with the same group before treatment

At the same time, ADSCs are easily obtained in large quantities from a wide range of sources, and their acquisition does not involve ethics and morality [16]. Although ADSCs effectively improve the survival rate of skin flap transplantation, the underlying mechanism is poorly understood. In this study, LRPSF model rats were treated with ADSCs, and in order to ensure the consistency of treatment, the skin flap was divided into three regions and injected bilaterally. Malondialdehyde (MDA) is a marker that reflects the degree of oxidative stress damage. It is produced when tissues are under oxidative stress. Thus, MDA reflects the level of reactive oxygen species (ROS) in the body. Superoxide dismutase (SOD) is an antioxidant enzyme that can effectively neutralizes reactive oxygen species generated by oxidative stress, thereby protecting the cells and tissues [17,18].

In this study, at 7 days after operation, the MDA level of rats in the study group was lower than that of rats in the control group, while the SOD activity was higher than that of the control group. On the 14th day after operation, MDA levels were decreased in both groups, while SOD activities of rats in the two groups were increased, with better conditions in the study group than in the control group. These results suggest that ADSCs enhanced survival of the flap by increasing the level of the antioxidant enzyme SOD, reducing MDA content, and alleviating oxidative stress damage. It is known that TNF-α and IL-6 are markers of inflammatory response which are closely related to the accumulation and infiltration of inflammatory factors [19,20]. The results of this study showed that the levels of TNF-α and IL-6 in the study group were significantly lower than those in the control group at days 7 and 14 post-operation. These results indicate that ADSCs have potential anti-inflammatory effects which may promote flap survival by inhibiting the inflammatory response and reducing the release of inflammatory factors. By observing the skin flaps, it was found that 3 days after the operation, the distal skin flaps of rats in the two groups had different degrees of necrosis, but the degree of necrosis in the study group was mild. On the 7th day after the operation, the degree of skin flap necrosis in the study group was significantly milder than that in the control group, and the flap survival rate was also significantly higher than that in the control group. These features may be related to reductions in oxidative stress and inflammatory response.

Study limitations

There are some limitations in this study. Due to certain constraints, skin tissue morphology and neovascularization after flap transplantation were not examined. These issues will be addressed in follow-up studies by providing relevant data on histological and immunohistochemical examinations.

CONCLUSION

This study has demonstrated that ADSCs inhibits inflammatory response and reduces the level of oxidative stress after long random pattern skin flap transplantation in rats, thereby enhancing blood supply to the flap transplantation area and improving the survival rate of the flap. These findings provide a basis for the development of ADSCs use in skin transplantation processes.

DECLARATIONS

Acknowledgements

Suzhou Science and Technology Plan Project Contract (no. SYS2019104).

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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REFERENCES

1. *Zhang X, Yao Y, Rao L, Qin Z, Zhang L, Xu Y, Chen Y, Yao J, Song D. Free sensate intercostal artery perforator flap for hand soft tissue reconstruction. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 2020; 34(4): 497-500.*
2. *Liu Y, Li X, Li R, Zhang J, Lu L. Reconstruction of complex nail matrix defect using the homodigital reverse fasciocutaneous flap. Medicine (Baltimore) 2018; 97(44): e12974.*
3. *He JB, Ma XY, Li WJ, Liu YY, Lin DS. Exenatide inhibits necrosis by enhancing angiogenesis and ameliorating ischemia/reperfusion injury in a random skin flap rat model. Int Immunopharmacol 2021; 90: 107192.*
4. *Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001; 7(2): 211-28.*
5. *Mazini L, Rochette L, Amine M, Malka G. Regenerative Capacity of Adipose Derived Stem Cells (ADSCs), Comparison with Mesenchymal Stem Cells (MSCs). Int J Mol Sci 2019; 20(10): 2523.*
6. *World Health Organization. Principles of laboratory animal care. WHO Chron 1985; 39: 51-56.*
7. *Dogan R, Metin Guler E, Kocyigit A, Bayindir N, Esrefoglu M, Mirasoglu BO, Yenigun A, Ozturan O. Comparison of the efficacy of multiple antioxidant and hyperbaric oxygen treatments in the prevention of ischemia and necrosis of local random McFarlane skin flap. J Tissue Viability 2021; 30(2): 196-206.*
8. *Tabary M, Aryannejad A, Noroozi N, Tavangar SM, Mohammad Jafari R, Araghi F, Dadkhahfar S, Dehpour AR. Ivermectin Increases Random-Pattern Skin Flap Survival in Rats: The Novel Role of GABAergic System. J Surg Res 2021; 259: 431-441.*
9. *Dwyer BJ, Jarman EJ, Gogoi-Tiwari J, Ferreira-Gonzalez S, Boulter L, Guest RV, Kendall TJ, Kurian D, Kilpatrick AM, Robson AJ, et al. TWEAK/Fn14 signalling promotes cholangiocarcinoma niche formation and progression. J Hepatol 2021; 74(4): 860-872.*
10. *Grootaert MOJ, Finigan A, Figg NL, Uryga AK, Bennett MR. SIRT6 Protects Smooth Muscle Cells From Senescence and Reduces Atherosclerosis. Circ Res 2021; 128(4): 474-491.*
11. *Fan W, Liu Z, Chen J, Liu S, Chen T, Li Z, Lin D. Effect of memantine on the survival of an ischemic random skin flap and the underlying mechanism. Biomed Pharmacother 2021; 143: 112163.*
12. *Casal D, Iria I, Ramalho JS, Alves S, Mota-Silva E, Mascarenhas-Lemos L, Pontinha C, Guadalupe-Cabral M, Ferreira-Silva J, Ferraz-Oliveira M, et al. BD-2 and BD-3 increase skin flap survival in a model of ischemia and Pseudomonas aeruginosa infection. Sci Rep 2019; 9(1): 7854.*
13. *Wang WZ. Investigation of reperfusion injury and ischemic preconditioning in microsurgery. Microsurgery 2009; 29(1): 72-9.*
14. *Masa I, Casado-Sánchez C, Crespo-Lora V, Ballestín A. Effects of Ischemic Preconditioning and C1 Esterase Inhibitor Administration following Ischemia-Reperfusion Injury in a Rat Skin Flap Model. J Reconstr Microsurg 2021; 37(3): 242-248.*
15. *Ding DC, Chang YH, Shyu WC, Lin SZ. Human umbilical cord mesenchymal stem cells: a new era for stem cell therapy. Cell Transplant 2015; 24(3): 339-47.*
16. *Li K, Shi G, Lei X, Huang Y, Li X, Bai L, Qin C. Age-related alteration in characteristics, function, and transcription features of ADSCs. Stem Cell Res Ther 2021; 12(1): 473.*
17. *Shojafar E, Soleimani Mehranjani M, Shariatzadeh SMA. Adipose derived mesenchymal stem cells improve the structure and function of autografted mice ovaries through reducing oxidative stress and inflammation: A stereological and biochemical analysis. Tissue Cell 2019; 56: 23-30.*
18. *Deng L, Du C, Song P, Chen T, Rui S, Armstrong DG, Deng W. The Role of Oxidative Stress and Antioxidants in Diabetic Wound Healing. Oxid Med Cell Longev 2021; 2021: 8852759.*
19. *Hu Q, Lyon CJ, Fletcher JK, Tang W, Wan M, Hu TY. Extracellular vesicle activities regulating macrophage- and tissue-mediated injury and repair responses. Acta Pharm Sin B 2021; 11(6): 1493-1512.*
20. *Seo E, Kang H, Lim OK, Jun HS. Supplementation with IL-6 and Muscle Cell Culture Conditioned Media Enhances Myogenic Differentiation of Adipose Tissue-Derived Stem Cells through STAT3 Activation. Int J Mol Sci 2018; 19(6): 1557.*