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Original Research Article

Effect of estrogen application timing on adipose tissue revascularization and immune regulation in rats after autologous fat transplantation

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Abstract

Purpose: *To study the effect of timing of estrogen application on adipose tissue revascularization and immune regulation in rats given autologous fat transplantation.*
Methods: *Ninety (90) healthy rats were selected for use in this study. The rats were randomly divided into a study group (n = 45) and control group (n = 45). After ovariectomy, the study group was given estrogen replacement therapy before autologous fat transplantation, while the control group was given estrogen replacement therapy after autologous fat transplantation. The rats were observed for 6 weeks after transplantation. Micro-vessel density, wet weight of transplanted fat, VEGF expression level, levels of M1 and M2 in macrophages, and macrophage infiltration rate were determined 40 days post-autologous fat transplantation.*
Results: *Forty days after autologous fat transplantation, microvessel density, wet weight of transplanted fat, expression level of VEGF and levels of M1 and M2 in macrophages were significantly higher in study group rats than in controls ($p < 0.05$). There was 80 % macrophage infiltration in the study group rats, while the corresponding control value (61 %) was significantly lower ($p < 0.05$).*
Conclusion: *Estrogen treatment before autologous fat transplantation in rats is helpful for the revascularization of adipose tissue, enhances vascular regeneration, promotes the survival of adipose tissue after transplantation, and activates immune cells. It also promotes the production of immune factors, and improves immunoregulation in adipose tissue. Thus, this therapeutic strategy may be useful in clinical practice, but further clinical trials are required.*

Keywords: *Timing of estrogen application, Autologous fat transplantation, Adipose tissue revascularization, Immune regulation*

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INTRODUCTION

Congenital defects in appearance, trauma or acquired tissue defects can be surgically repaired. In clinics, the traditional surgical method is surgical removal of fat block or dermal fat block transplantation. However, the beneficial effect of surgical repair is low due to the traditional surgical trauma which increases surgical scar, and may cause defects [1]. With continuous advancements in modern medical technology and medical equipment, autologous

fat transplantation has been applied in clinical burn plastic surgery instead of traditional transplantation, with good application effects [2]. Autologous fat transplantation is a surgical method used to improve the morphology of the defective parts of the body by injecting the fat-rich parts of the body into the defective recipient area that needs to be changed, after special treatment using negative pressure liposuction [3].

Autologous fat transplantation adopts injection method, resulting in reduced trauma, absence of obvious postoperative scar in donor and recipient area, natural shape of recipient area, repeatability of injections, low surgical cost, and easy acceptability by patients. The survival of adipose tissue after autologous fat transplantation is crucial. When the transplanted adipose tissue is fixed, the distribution of blood vessels in the affected area and postoperative treatment affect the survival of the transplanted tissue, and appropriate drugs are required to inhibit apoptosis of adipose tissue [4].

Estrogen, a female hormone secreted by the ovaries and placenta, is a substance that promotes the maturation of sexual organs in female animals and plays an important physiological role [5].

Although this type of transplantation is popular in clinical practice, survival of transplanted fat is controversial [6]. Clinical studies have shown that estrogen regulates the metabolism of adipose tissue, acts on autologous fat transplantation, and effectively inhibits the apoptosis of adipose tissue, making it beneficial for postoperative recovery and survival of adipose tissue after transplantation. However, there are limited studies on the influence of adipose tissue revascularization and immune regulation [7].

In this study, the effect of timing of estrogen application on adipose tissue revascularization and immunoregulation in autologous rats after fat transplantation was investigated [8].

EXPERIMENTAL

Animals

Ninety healthy female rats with average age of 3 months and weights of 214-256 g were selected and reared in a university experimental center at 21 ± 3 ℃ and 42 ± 4 % humidity. The rats were fed with water and feed for 1 week. The 90 female rats were divided into observation group and control group. The two groups were comparable. This research was approved by the Animal Ethical Committee of Taizhou Hospital of Zhejiang Province approval no. 20200932, and was performed according to the guidelines of "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [9].

Treatments

Study group

Rat ovarian tissue was removed and estrogen replacement therapy was applied prior to autologous fat transplantation. Ovariectomy was done under an anesthetic given via intraperitoneal injection after 1 week of feeding. In this process, the hair on the back of each rat was shaved and the rat was fixed on an operating table. The back skin of each rat was disinfected with iodavor and a sterile tissue was laid on top. Then, using a surgical blade, a 1-cm incision was made about 1.5 cm on both sides of the spine to separate the subcutaneous tissue from fascia. The tissue near the spine was clamped with vascular forceps, the rat was ligated, and then the ovarian tissue was excised.

After surgery, the tissue was washed with normal saline, and a penicillin suspension was sprayed around the surgical wound, followed by suturing of the wound. For several days during postoperative recovery, the rats were monitored to prevent postoperative infection. Before the autologous fat transplantation surgery, the rats were treated with estradiol benzoate instead of estrogen, and after 40 days of treatment, the rats were subjected to autologous fat transplantation surgery under full anesthesia administered via peritoneal injection. After 5 min, the shaved rats were fixed on the operating table and prepared for surgical disinfection in the groin area according to the principle of sterile treatment. Then, a 1-cm incision was made in the bilateral groin of each rat, and after tissue separation, about 3 ml of fat was cut off with a pair of scissors, and the surgical wound was rinsed with saline and sutured.

Finally, the excised 3 ml of adipose tissue was put into a sterile vessel, and the mucosa on the adipose tissue surface was rinsed in saline. The adipose tissue was cut into 1.5-mm fat sections and centrifuged at 700 rpm for 3 min. Thereafter, 1.5 mL of the supernatant was extracted with a 2-ml syringe. The fat was injected into the back of each rat using a high-pressure injection, and the rats were maintained for 40 days after surgery. After 40 days of rearing, the rats were sacrificed by exposure to excess anesthesia. The transplanted adipose tissue was removed, and half of the adipose tissue was cut into sections

which were stained with CD31 in order to measure microvessel density. The other half was visualized using immunohistochemical staining.

Control group

Ovarian tissues of rats were excised and estrogen was used as replacement therapy after autologous fat transplantation. Using the same postoperative method as was applied in the observation group, the rat ovarian tissues were removed, and no estrogen replacement treatment was continued for several days. After several days of observation, the rats were subjected to autologous fat transplantation, and estrogen was replaced with estradiol benzoate. After 40 days, the transplanted adipose tissue was removed. Half of the tissue was examined as sections, while the other half was examined using immunohistochemical staining.

Evaluation of parameters/indicators

Microvascular density

The prepared sections were placed under an optical microscope to examine tissue and microvascular status, while vascular condition was examined according to the vascular density counting method. The higher the microvascular density, the better the vascular condition.

Fat survival

This was determined based on the wet weight of transplanted fat. The heavier the wet weight of transplanted fat, the higher the fat survival. The expression level of VEGF in fat transplantation was compared.

Immunoregulation

Based on macrophage M1 and M2, macrophage infiltration (F) was calculated as in Eq 1.

$$F (\%) = M1/M2 \quad \ldots\ldots\ldots (1)$$

Statistical analysis

The SPSS 20.0 software package was used for statistical analysis of data in this study. All measurement data in line with normal distribution are presented as mean ± SD. Independent sample *t*-test was used for inter-group comparison, while paired sample *t*-test was used for comparison before and after treatment. Counting data are expressed as percentage, and χ^2 test was used for comparison between groups. $P < 0.05$ was considered statistically significant.

RESULTS

Microvascular density

Forty days after autologous fat transplantation, microvascular density was significantly higher in the study rats than in control rats ($p < 0.05$; Table 1).

Table 1: Microvascular density values at the time of estrogen application (mean ± SD, n = 45)

Group	Microvascular density (per/mm²)
Study	43.5287±3.1543
Control	26.3245±4.6423
t	20.563
P-value	<0.001

Wet weight of transplanted fat

Forty days following transplantation, the transplanted fat wet weight was raised in the study rats, relative to control rats ($p < 0.05$), as shown in Table 2.

Table 2: Wet weight of transplanted fat in each group (mean ± SD, n = 45)

Group	Wet weight of transplanted fat (g)
Study	0.1145±0.0465
Control	0.0689±0.0145
t	6.280
P-value	<0.001

VEGF expression levels after fat transplantation

Forty days post-surgery, the expression level of VEGF was markedly up-regulated in study rats, relative to control rats ($p < 0.05$; Table 3).

Table 3: Comparison of VEGF expression levels in transplanted fat between the two groups (mean ± SD), n = 45)

Group	Expression level of VEGF
Observation	0.0054±0.0008
Control	0.0025±0.0002
t	23.591
P-value	<0.001

Levels of macrophage M1 and M2 and macrophage infiltration

Forty days after autologous fat transplantation, the levels of macrophage M1 and M2 were significantly higher in study rats than in control rats. Moreover, infiltration of macrophages was significantly higher in study rats (80 %) than in control rats (61%) ($p < 0.05$; Table 4).

Table 4: Levels of macrophage M1 and M2, and % macrophage infiltration in the two groups (mean ± SD)

Group	n	Macrophage M1	Macrophage M2	Infiltration (%)
Study	45	6.36±2.56	4.58±1.23	80
Control	45	4.17±1.23	2.43±0.25	61
t		5.173	11.491	
P-value		<0.001	<0.001	

DISCUSSION

Traditional fat transplantation surgery leads to great surgical trauma, long postoperative recovery time and many complications, and the gray effect after surgery is not usually obvious [10]. With improvements in medical technology, autologous fat transplantation has been widely used in clinics, with remarkable effects when applied in plastic surgery for burns application. Under local anesthesia, electric negative pressure liposuction method is used to extract fat which is converted into fat injections comprising purified fatty particles which can be injected into the relevant area. The surgical site is small, without obvious scars, and the effect is remarkable [11].

Estrogen is a female sex hormone which is the most active amongst all the important female hormones, including estradiol, estradiol and estriol. The dynamic balance between estradiol and estriol in blood is regulated by the hypothalamus, resulting in regulation of body metabolism, and it has important physiological functions [12]. Estrogen expression is carried out through estrogen receptor (ER) which is located in cell membrane, nucleus and cytoplasm, and it is divided into α receptor and β receptor which are key receptors of estrogen [13].

Several studies have shown that estrogen effectively regulates the metabolism of adipose tissue, inhibits the apoptosis of adipose cells, and plays an effective role in autologous fat transplantation. However, there are limited studies on the effect of estrogen application timing on the revascularization of adipose tissue and immunoregulation in autologous fat transplantation [14]. This study showed that 40 days after autologous fat transplantation, microvascular density was markedly higher in observation rats than in control rats, indicating that estrogen replacement therapy before autologous fat transplantation was beneficial to the revascularization of adipocytes.

Forty days after autologous fat transplantation, the wet weight of the transplanted fat was heavier in study rats than in control rats, indicating that estrogen replacement therapy was beneficial for increasing the wet weight of fat and the survival of adipose tissue. Forty days following transplantation, the expression level of VEGF was markedly up-regulated in the observation rats, relative to control rats. This indicates that the combination of estrogen and receptor improved VEGF level and contributed to the promotion of angiogenesis.

The effect of estrogen on autologous fat transplantation has been reported in literature, but there are very few studies on the effect of estrogen on immunoregulation in adipose tissue [15]. Macrophages, as immune cells, are a kind of white blood cells located in tissues and differentiated from monocytes in the blood after escaping from blood vessels. The macrophages are crucial in the study of phagocytosis, cellular immunity and molecular immunology [16].

Macrophages comprise M1 type and M2 types. The M1 macrophages are classically activated macrophages that elaborate pro-inflammatory cytokines and chemokines, and take part in the positive immune response where they play the role of immune surveillance [17]. The M2 macrophages have weak antigen presentation ability; they down-regulate the immune response by secreting inhibitory cytokine IL-10, and play important role in immune regulation [18].

In this study, 40 days after autologous fat transplantation, the levels of M1 and M2 macrophages were markedly higher in observation rats than in control rats, and percentage infiltration of macrophages was higher in the former (80%) than in the latter (61%), with statistically significant difference. Therefore, estrogen replacement therapy in the early stage of fat transplantation effectively improved the levels of anti-inflammatory macrophages, activated immune cells to produce immune factors, and suppressed ischemia and hypoxia environment, thereby enhancing immunoregulation.

CONCLUSION

Estrogen therapy before autologous fat transplantation is conducive to the revascularization of adipose tissue. It increases wet weight and improves the survival of adipose tissue as well as play an immunoregulatory

role. However, because of limited number of animals used, and brief observation period, the effects of estrogen application timing on adipose tissue revascularization and immune regulation during autologous fat transplantation need to be further investigated using larger number of samples.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Lili Zhu designed the study, supervised the data collection, and analyzed the data. Weigang Lin interpreted the data and prepared the manuscript for publication. Mirong Liao and Yunyan Zhang supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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