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The effect of penile urethral fat graft application on urethral angiogenesis

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Summary

Background
Autologous fat grafts are rich in adipose-derived stem cells, providing optimal soft-tissue replacement and significant quantities of angiogenic growth factor. Although fat grafts (FG) are used in several clinical conditions, the use of FG in urethral repairs and the effects of FG to urethral repairs have not yet been reported.

Objective
An experimental study was performed to evaluate the effect of FG on urethral angiogenesis and tissue growth factor (GF) levels.

Study design
Sixteen Wistar albino, adult, male rats were allocated into two groups: the control group (CG) ($n = 8$) and the experiment group (EG) ($n = 8$). After anesthetization of all rats, 3-mm vertical incisions were made on the urethras, and then sutured with interrupted 5/0 vicryl sutures. The operations were performed under a stereo dissecting microscope under magnification (×20). In the CG, no additional procedure was performed. In the EG after the same surgical procedure, 1 mm^3 FG was removed from the inguinal region by sharp dissection with a knife. The grafts were trimmed to 1 × 1 mm dimensions on millimeter paper. The FGs were placed on the repaired urethras. The skin was then closed. Samples from urethral and penile skin were taken 21 days after surgery in both groups. Density and intensity of staining with vascular-endothelial GF (VEGF), VEGF-receptor, and endothelial-GF receptor (EGFR) in the endothelial and mesenchymal cells of the penile urethral vessels were immunohistochemically evaluated. Data obtained from immunohistochemical evaluations were analyzed with SPSS 15.0. The *P*-values lower than 0.05 were considered as significant.

Results
Density of VEGF staining was significantly decreased in the vascular endothelium of the EG compared to the CG ($P < 0.05$). Density of the EGFR staining was significantly decreased in the vascular endothelium of the EG compared to the CG ($P < 0.05$) (Table). Intensity of VEGF, VEGF-R and EGFR staining was not significantly different between the two groups. There were no significant differences between groups regarding to VEGFR staining and mesenchymal examination.

Discussion
Decreased density was found in the VEGF staining in the vascular endothelium. This could be explained by the day that the tissues were harvested or because autologous fat grafts might cause decreased growth factor levels, which is contrary to the literature data.

Conclusion
Fat grafting has an immunohistochemical effect on the growth factor levels that are related to angiogenesis after urethral repair. It is difficult to make a firm conclusion about the role of fat grafting on urethral healing. Therefore, future studies are needed to see if FG can be used as an alternative to other procedures in order to avoid complications.

Table The median values of density grades of VEGF, VEGF-R, and EGF staining in the vascular endothelium (interquartile ranges within brackets).

	Control group	Experimental group
VEGF	2.0 (1.75–2.0) α	1.0 (0–1.0) α
VEGF-R	1.50 (0–2.25)	0.50 (0–1.0)
EGF	2.0 (1.75–2.0) β	1.0 (0–1.25) β

α, β, $P < 0.05$.
vascular-endothelial growth factor: VEGF, VEGF-receptor: VEGF-R, endothelial-growth factor receptor: EGF.

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Introduction

Reconstruction of the urethra is one of the most challenging issues in pediatric urology. It is mostly required to treat congenital urinary anomalies — mostly hypospadias — and less commonly in traumatic defects or urethral strictures [1]. A variety of surgical techniques for urethral repair, especially in hypospadias, have been introduced in order to get satisfying results after surgery. Besides some well-known issues, including tension-free anastomosis, gentle tissue handling, using absorbable suture materials and covering with well-vascularized tissue, several surgical grafts or flaps have also been developed to minimize the complication rates [2,3]. Despite all of the advances in surgical techniques, complications after urethral repair are still problematic, indicating the presence of congenital or structural anomalies in the penile urethra and prepuce of hypospadiac children [4—7].

Autologous fat transfers have been widely used for improving the quality of underlying soft tissue, especially in plastic surgery [8]. These fat grafts (FG) can be injected into another tissue/organ as a free graft. They are rich in adipose-derived stem cells, providing optimal soft-tissue replacement. Fat grafts have also been reported to contain significant quantities of angiogenic growth factors, such as vascular endothelial growth factor (VEGF), which have a possible role in wound healing [8,9]. Although these grafts are used in several clinical conditions, the use and effects of FG in urethral repair have not yet been reported. Therefore, an experimental study was performed to evaluate the effect of FG on urethral angiogenesis and tissue growth factor (GF) levels.

Materials and methods

The experiments were performed after approval by the Local Ethical Committee and the study was supported by Kırıkkale University Scientific Research Council (KU-2011/47).

Animals and experimental design

Sixteen Wistar albino, male, adult rats, weighing between 250 and 300 g, were used in the study. They were housed in standard cages under the same environmental conditions. The rats were kept at 22 °C room temperature and 12-h day/night cycle. They were provided with tap water and standard food *ad libitum*.

The rats were randomly divided into two groups: control group and experimental group. They were anesthetized with intraperitoneal ketamine hydrochloride (50 mg/kg, Ketalar, Eczacıbası, Istanbul, Turkey). In all animals, after anesthetization, 3-mm vertical incisions were performed on the urethra, and then they were sutured with interrupted 5/0 vicryl sutures. In the control group (CG) ($n = 8$), no additional procedures were done. In the experiment group (EG) ($n = 8$), 1 mm^3 FG was taken from the inguinal region and placed onto the repaired urethra.

In both groups, samples from the urethra and penile skin were taken 21 days after surgery. Density and intensity of staining with vascular-endothelial GF (VEGF), VEGF-receptor, endothelial-GF receptor (EGFR) in the endothelial and mesenchymal cells of the penile urethral vessels were immunohistochemically evaluated.

Data obtained from the immunohistochemical evaluations were analyzed with SPSS 15.0. The distribution between groups was analyzed with the Kruskal—Wallis test and the difference between two groups was evaluated with the Mann—Whitney U test. The *P*-values lower than 0.05 were considered as significant.

Operation and postoperative care

The rats were placed in the supine position. Their genitalia were prepared with poviodine-iodine. The operations were performed under stereo dissecting microscope (Amscope, China) magnification (×20). A 23-gauge angiocath was used to catheterize the urethra. The penile skin was incised circumferentially at the level of the mucosa and hairy skin junction on the penis. In order to reach the urethra it was then degloved up to the glans. A 3-mm length vertical incision was performed on the urethra and then sutured with interrupted 5/0 vicryl sutures (Fig. 1).

In the experimental group, the FG was then harvested from the inguinal fat pad. The inguinal regions of the animals were shaved and prepared with poviodine-iodine. The fat grafts were taken by sharp dissection with a knife. The grafts were prepared on cotton gauze before implanting onto the urethras. The excessive connective tissue was cleaned and grafts were trimmed to 1 × 1 mm dimensions on millimeter paper in order to standardize them (Fig. 2). The FGs were placed onto the repaired regions of the urethras. The skin was closed with interrupted 5/0 silk sutures.

The repaired regions on the urethras were marked with non-absorbable sutures in all animals. All animals urinated well after the procedures. They were sacrificed on 21st postoperative day for sampling. The penises were harvested and fixed in 10% formalin.

Immunohistochemical evaluation

From paraffin embedded blocks, 4—6 μm sections were obtained. Sections were immunohistochemically stained using an automatic staining machine (Bond™ System, Leica Microsystems GmbH, Wetzlar, Germany) with a biotin-free Bond™ Polymer Define Detection System (Leica Microsystems GmbH, catalog no. DS9800, Germany). Vascular endothelial growth factor antibody (VEGF-Abcam; 1/50), vascular endothelial growth factor receptor-1 antibody (VEGFR1-Abcam; 1/50) and endothelial growth factor receptor antibody (EGFR, Abcam; 1/80) were used as primary antibodies.

Ethylenediaminetetraacetic acid (EDTA) was used as the epitope retrieval solution for VEGFR-1 and EGFR antibodies. Citrate buffered solution (pH = 6) was used as the antigen retrieval solution for VEGF antibodies. 3,3′-Diaminobenzidine (DAB) was used as the chromogen. Slides were washed between all steps in phosphate-buffered saline with a pH of 7.4. Placental tissues were used as a positive control for VEGF and VEGFR-1 and breast carcinoma sections were used for EGFR. Phosphate-buffered