European Urology

European Urology 47 (2005) 417–421

Comparison of Angiogenic Activities after Urethral Reconstruction Using Free Grafts in Rabbits☆

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Accepted 19 October 2004

Available online 10 November 2004

Abstract

Objective: To determine the most suitable type of graft-free penile skin grafts or mucosal grafts from bladder or buccal regions - for urethral reconstruction in an animal model, as evaluated on the basis of angiogenic activity.
Methods: Twenty-two male White New Zealand rabbits were randomly divided into four groups. In the control group (group O, $n = 4$) a simple urethrotomy and closure was performed, whereas a ventral urethral defect was created in groups A, B, and C and then bridged using the following onlay patches: free penile skin (group A, $n = 6$), buccal mucosal graft (group B, $n = 6$), and bladder mucosal graft (group C, $n = 6$). On the 21st postoperative day, the animals were sacrificed and the retrieved implants were subjected to macroscopic and microscopic analysis. The angiogenic activity was assessed with immunohistochemistry, using the anti-CD31 MoAb and the phosphatase antialkaline phosphatase procedure. The native vascularity of penile skin as well as buccal and bladder mucosa was assessed in rabbits from group O ($n = 3$). Statistical analysis was performed using the one-way ANOVA.
Results: The angiogenesis in a magnification of $\times 200$ in groups O, A, B, and C was 34.1 ± 4.1 (mean $\pm$ SD), 61.7 ± 6.4, 94.3 ± 6.4, and 91.5 ± 7.2 vessels per optical field, respectively. There were, statistically significant differences ($p < 0.001$) between groups A and B and between groups A and C, but not ($p > 0.05$) between groups B and C. The native vascularity of penile skin, buccal mucosa and bladder mucosa was 23.3 ± 3.0, 24.6 ± 3.7 and 17.0 ± 2.6 vessels per optical field, respectively.
Conclusion: The viability of mucosal grafts from bladder or buccal regions is better than that of a free penile graft because of higher angiogenic activity. Although the mucosal grafts showed the same angiogenic activity, the buccal mucosa graft is preferable because of its easier harvesting.

Keywords: Angiogenesis; Angiogenic activity; Bladder mucosa graft; Buccal mucosa graft; Free skin penile graft; Graft; Rabbit; Neovascularization; Urethral defect; Urethral reconstruction

1. Introduction

Urethral reconstruction under several pathologic conditions, such as strictures, traumatic defects, epispadias, and mainly in hypospadias, is one of the oldest problems in reconstructive surgery and one of the

☆ Presented at the XIXth EAU Congress,Vienna, Austria, March 24–27, 2004.

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doi:10.1016/j.eururo.2004.10.014

greatest surgical challenges for the surgeon. A variety of donor tissues have been used both experimentally and clinically for urethral repair, including free penile or preputial graft [1], hairless-skin grafts [2], bladder mucosal graft [3], buccal mucosal graft [4], tunica vaginalis graft [5], peritoneal graft [6], vein graft [7], intestinal submucosa graft [8], colonic mucosa graft [9], and deep facia graft [10]. Autologous grafts of cultured epithelium and acellular collagen matrix have also been described for urethral repair [11,12]. The use of so many different graft types indicates that the ideal graft material has not been identified.

In clinical practice, the grafts that are usually used for urethral reconstruction are penile or preputial skin graft and mucosal grafts from bladder or buccal regions [13]. Therefore, this study was designed to determine which of these graft types was the most suitable for urethral reconstruction, on the basis of angiogenic activity. We use a rabbit model of the urethral defect because of the similarity of the anatomy and size of human and rabbit penises making it ideal for experimental surgical operations [14].

2. Methods

2.1. Animals

Twenty-two male White New Zealand rabbits, 6 months of age and weighing 3.8–4.2 kg, were used in this study. They were housed individually in wire cages under controlled environmental conditions (20–22 °C room temperature, 50–60% relative humidity, 12-hour-light/12-hour-dark cycle). The animals were provided with 125 g of commercial pelleted diet (certified rabbit chow #51, EL.VI.Z., Xanthi, Greece) per day and tap water ad libitum. The animals remained unfed for about 24 hours before surgery, while the water was permitted for up to 3 hours before the beginning of the experiment.

2.2. Experimental design

The animals were randomly divided into four groups. In the control group (group O, $n = 4$) a simple urethrotomy and closure was performed, whereas a ventral urethral defect was created in groups A, B, and C and then bridged using the following onlay patches: free penile skin (group A, $n = 6$), buccal mucosal graft (group B, $n = 6$), and bladder mucosal graft (group C, $n = 6$). On the 21st postoperative day, the animals were sacrificed and their penises were excised and subjected to macroscopic and immunohistochemistry analysis.

The experimental protocol was approved by the Ethics Committee of the local Veterinary Service since it was in compliance with Directive 86/609/EE.

2.3. Anesthesia

The animals were sedated by injection of xylazine hydrochloride (Rompum 2%, 5 mg/kg, i.m.) and atropine sulfate (0.04 mg/kg, i.m.). Twenty minutes later, general anesthesia was induced by the injection of ketamine hydrochloride (Ketaset 10%, 50 mg/kg, i.m.). Anesthesia was maintained either by intermittent doses of ketamine (groups O and A) or by isoflurane administration (0.5–1% in oxygen) after the trachea was intubated (groups B and C). In the latter cases, anesthesia equipment and a pressure ventilator (ADS 1000 Veterinary Anesthesia Delivery System/Engler, FL, USA) operating in a non-rebreathing circuit was used which, was set to provide 12 l of anesthetic gas/min, 30 breaths/min, 15 cmH_2O peak inspiratory pressure, and inspiration triggering at -2 cmH_2O.

2.4. Operation

The rabbits were placed at a supine position and their genitalia were prepared with povidone-iodine and draped with a sterile technique. A 10-French catheter was inserted into the urethra. After exposing the urethra with sharp dissection, a ventral segment measuring 1.0×0.5 cm (approximately one-third of the urethral circumference) was excised in groups A, B, and C approximately 3 cm from the distal urethral meatus with the penis on partial stretch. In the group O, a simple urethrotomy and closure was performed (7-0 Vicryl as a continuous suture).

The techniques of graft harvesting from penile skin and bladder or buccal mucosal regions were as follows. Animals that underwent a penile graft (group A) had the graft harvested from the non-hair-bearing penile skin located on the dorsal aspect. In group B a full thickness of buccal mucosal graft (from the inner cheek) was procured using a knife and sharp scissors. In group C the abdomen was scrubbed with povidone-iodine solution and draped sterilely. The bladder was exposed through a midline abdominal incision and the detrusor muscle was carefully dissected. Four tagging sutures of 6-0 Vicryl were placed at the corners of the exposed mucosal segment, after which, the isolated section of bladder mucosa was excised. The donor sites were closed with a running 5-0 Vicryl rapid suture.

The grafts were stretched and fixed on a board so that excess subcutaneous connective tissue could be removed, and they were trimmed and placed over the urethral defect as an onlay patch. The urethral repair was accomplished using 7-0 Vicryl as continuous suture. Multiple nonabsorbable marking sutures (Prolene) were placed at the anastomosis margins. One additional layer of subcutaneous tissue was used to cover the grafts with 7-0 Vicryl. The skin was used to cover the graft, and it was reapproximated with 6-0 Vicryl interrupted sutures.

2.5. Postoperative care

An Elizabethan collar was placed until the urinary catheters were removed on the 7th postoperative day. All animals received antibiotic treatment (keftazidime, 10 mg/kg/8 hours, i.m.) for 7 days.

On the 21st postoperative day, the animals were sacrificed by isoflurane overdose administered via a facemask. Their penises were removed and fixed in 10% formalin.

2.6. Macroscopic examination

A longitudinal incision was made along the ventral aspect of the urethra lumen. The lumens were measured and examined for stricture and diverticula formation.

2.7. Immunohistochemistry

Serial sections of the grafts were taken from within the area outlined by the marking of sutures and processed for microscopic examination with histochemical staining. The angiogenic activity was examined in paralled section from paraffin embedded tissues with immunohistochemistry, using the anti-CD31 MoAb and the phosphatase antialkaline phosphatase (APAAP) procedure. In