THE TUNICA VAGINALIS DORSAL GRAFT URETHROPLASTY: EXPERIMENTAL STUDY IN RABBITS

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ABSTRACT

Purpose: We created an experimental model of urethral defect and then repaired it using a tunica vaginalis graft applied on the dorsal surface of the urethra. We studied the histological and radiological characteristics of free tunica vaginalis graft urethroplasty.

Materials and Methods: In 20 New Zealand rabbits a dorsal urethral defect was created by excising a portion of the dorsal urethral surface. The tunica vaginalis graft was placed dorsally over the corpora cavernosa and tied with 4 interrupted sutures. The mucosal margin of the urethral defect was sutured to the graft using 6–zero polydioxanone sutures in continuous fashion. The animals were divided into 4 equal groups and were sacrificed 14 days, and 4, 8 and 12 weeks after surgery, respectively. A retrograde urethrogram was done at autopsy. The penis was sent for histological analysis and an experienced pathologist evaluated the severity of acute and chronic inflammation, foreign body reaction and scar formation.

Results: There were no deaths related to the procedure and no intraoperative complications. All rabbits voided spontaneously after surgery. Retrograde urethograms showed no fistula or stricture. As time after surgery increased, the signs of inflammation response disappeared, and the orientation of collagen fibrils and smooth muscle fascicles resembled that of a normal urethra. The mesothelial lining of the tunica vaginalis gradually became replaced by a more stratified epithelial lining, similar to the urothelial lining of the native urethra.

Conclusions: In the current study we noted that a tunica vaginalis graft placed dorsally can be a successful urethral substitute in the animal model.

KEY WORDS: urethra, rabbits, transplantation

Urethral reconstruction following failed hypospadias repair or posttraumatic chronic stricture requires adequate amounts of tissue. Various donor tissues have been used clinically for urethral repair, including vascularized skin flaps from the prepuce, scrotum or penile shaft, full-thickness free skin grafts, vesical or buccal mucosa grafts, and tunica vaginalis, ureter, artery, vein and appendix tissue.

Actually, vascularized skin flaps are the preferred material for use in urethral reconstructions when available, mainly for primary urethral repairs. Unfortunately in many cases they may not be available or sufficient in patients with inadequate penile skin due to previous failed hypospadias surgery, circumcision or an unusual urethral defect.

We created an experimental model of urethral defect and then repaired it using a tunica vaginalis graft applied on the dorsal surface of the urethra over the corpora cavernosa. We studied histological and radiological outcomes after repairing this experimentally induced urethral defect.

MATERIALS AND METHODS

We chose the rabbit model because it has some important advantages, including ease of manipulation, ready access to a urethra that is simple to work with and well-known urethral histology. A total of 20 New Zealand White rabbits at approximately 8 weeks old and weighing 2.5 to 3.0 kg were acclimated in the Experimental Research Animal Surgery Department for 1 week before the study. Two rabbits served as untreated controls to assess the normal histological outcome. The experimental protocol was reviewed and approved by the Local Animal Research Committee.

Two animals died in the immediate postoperative period. Each rabbit was unable to awaken and had cardiac and pulmonary insufficiency.

The rabbits were anesthetized intramuscularly with ketamine hydrochloride (30 mg/kg) and xylazine (5 mg/kg). Ceftriaxone was administered before surgery and daily for 3 subsequent days. After a adequate level of anesthesia was achieved the penis was anesthetized with xylacaine and a 10Fr urethral catheter was inserted. Under sterile conditions the penis was released by dividing the perineal skin web between the ventral aspect of the penis and the anus. The penile urethra was exposed through a midline skin incision. All the animals were operated under optical magnification (3.5×).

The urethra was carefully dissected and mobilized off of the tunica albuginea (fig. 1, A). After exposing the urethra a dorsal segment that was 2.0 × 1.0 cm (approximately half of the urethral circumference) was excised in all rabbits (fig. 1, B).

The skin and dartos of the left hemiscrotum were incised to deliver the left testis in its tunica vaginalis covering. The tunica was incised longitudinally along the anterior surface and freed from its testicular attachment. The graft was obtained by sharp dissection with fine scissors (fig. 1, C). The donor site was closed with 5–zero running catgut sutures.

The tunica vaginalis graft was placed dorsally over the corpora cavernosa and tied with 4 interrupted polydioxanone 6–zero sutures (fig. 1, D). The mucosal margin of the urethral defect was sutured to the graft using 6–zero polydioxanone
sutures in continuous fashion. The visceral surface of the tunica vaginalis was always placed as the lumen of the reconstructed urethra. The skin was closed with a running 5–zero chromic catgut stitch. Neither stent nor dressing was used. Figure 2 shows the operative technique.

The animals were recovered and returned to our chronic care facility. They were examined daily to monitor wound healing.

Experimental animals were divided into 4 equal groups of 4 each. They were sacrificed 14 days, and 4, 8 and 12 weeks after surgery, respectively. A retrograde urethrogram was done at autopsy. At the scheduled sampling time the animals were sacrificed by injection with an overdose of ketamine. The entire penis was examined and removed. The penises were fixed in 10% formaldehyde and transverse sections were cut to produce segments of 5 mm each, processed into paraffin blocks, serially sectioned and stained with hematoxylin and eosin, Masson’s trichrome and Picrosirius red. An experienced pathologist (RD) examined the specimens and evaluated the severity of acute and chronic inflammation, foreign body reaction and scar formation. The degree of inflammation was based on a score of 0 to 4, that is 0—no inflammation to 4—the most extensive reaction. Acute inflammation was characterized by the presence of polymorphonuclear cells, chronic inflammation was characterized by the presence of monocytes and lymphocytes, and foreign body reaction was characterized by the presence of macrophages and multinucleated foreign body giant cells.

Masson’s trichrome and Sirius red stains were used to localize collagen. With Masson’s trichrome stain nuclei stain deep mauve to black, cytoplasmic elements stain red and blue, muscle stains red and collagen-mucus stains green. To evaluate collagenous types I and III on slides stained with Sirius red a polarizing filter was used. Under this filter type I fibers appear reddish-yellow and strongly birefringent, and type III fibers appear green.¹

RESULTS

There were no deaths related to the procedure and no intraoperative complications or difficulties were associated with tunica vaginalis harvesting. All rabbits voided spontaneously after surgery. The macroscopic appearance of the operated penises was normal. When the rabbits were sacrificed, the urethra was easily calibrated using a 10Fr Foley catheter in all. Retrograde urethrogram confirmed the maintenance of a wide urethral caliber without any signs of stricture or extravasation (fig. 3).

Two weeks after surgery a moderate infiltration of polymorphonuclear cells was observed, representing an acute inflammatory reaction. As time after surgery increased, the

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signs of inflammation response disappeared, and the orientation of collagen fibrils and smooth muscle fascicles resembled that of a normal urethra. Complete disappearance of the polymorphonuclear cells, representing resolution of the acute inflammatory reaction, was evident by 4 weeks postoperatively. The table shows inflammation scores.

Eight and 12 weeks after surgery signs of inflammatory response disappeared and collagen fibrils showed early orien-
Inflammatory response following tunica vaginalis urethroplasty

<table>
<thead>
<tr>
<th>Time After Surgery</th>
<th>Acute Inflammation</th>
<th>Chronic Inflammation</th>
<th>Fibrosis/Scarring</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 Days</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4 Wks</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>8 Wks</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>12 Wks</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Score range 0—not present, 1—minimal, 2—mild, 3—moderate and 4—extensive.

ization. There was minimal evidence of fibrosis in the urethras at the latter period. Microscopically the junction of the graft and normal urethra was identifiable in all groups (figs. 4 and 5).

The mesothelial lining of the tunica vaginalis gradually became replaced by a more stratified epithelial lining, similar to the urothelial lining of the native urethra. After 4 weeks the junction between the native epithelium and the peritoneum lined graft was difficult to detect. The tunica vaginalis grafts covering epithelium were thin, consisting of 3 or 4 cell layers of transition cell-like epithelium, whereas the normal epithelium was composed of 8 to 10 cell layers (fig. 6).

**DISCUSSION**

Defects in the male urethra caused by congenital malformations or traumatic injuries have created a need for tissues that can serve as adequate urethral substitutes. Among the various alternatives available for urethral reconstruction, vascularized grafts of nonhair bearing skin probably are the most popular. However, many problems still remain with the current methods, making it a field of further exploration for reconstructive urologists.

Theoretically bladder mucosa grafts may be well suited for contact with urine. However, bladder mucosa has been associated with many complications, including meatal stenosis, prolapse and a granulomatous reaction at the urethral meatus. Kinkead et al reported results in 95 patients who underwent complex urethral reconstruction using bladder mucosa grafts. There were complications in 63 patients (66%), while 21 required major revision. Also, bladder mucosa grafts may be difficult to harvest in a child who has undergone a previous bladder operation or in patients with neurogenic dysfunction.

To overcome these problems Bürger et al studied the use of buccal mucosa grafts for urethroplasty in 2 dogs before performing the operation in 6 patients. A fistula and meatal stenosis developed in 3 and 1 of the 6 patients, respectively. Actually, buccal mucosal grafts have proved to be a versatile substitute for strictures attributable to a wide range of causes. Fichtner et al reported a large clinical series with excellent success rates. They retrospectively evaluated 132 patients who received a buccal mucosa onlay graft for hypospadias repair and analyzed a subgroup of 49 with longer than 5-year followup available. The overall complication rate was 24% and almost all complications occurred during the first 12 months.

Recently our group described an original approach to treating primary complex hypospadias using the buccal mucosa to reconstruct the urethral plate in such patients when preservation of the urethral plate is not possible.

Tunica vaginalis is an extension of the peritoneum, described as a fibrovascular connective tissue lined by mesothelium. It has been observed that the peritoneum undergoes conversion into a urothelium-like lining when placed into the urinary tract.

The initial report of tunica vaginalis grafts was in 1979 by Das and Maggio. Grafhs were placed in dogs after excising a segment of tunica albuginea. The animals were sacrificed at 2, 4 and 6 months, respectively. At 6 months it was noted that the tunica graft was indistinguishable histologically from the surrounding tunica albuginea. No contracture or scarring of the graft was noted. The first clinical use was described in 1985 by Perlmutter et al. Tunica vaginalis grafts were used for correcting chordee in 11 boys with satisfactory short-term results.

In urethral reconstruction tunica vaginalis tissue has been used experimentally as pediculated and free grafts. A tunica vaginalis graft is easily obtained and it is a technically simple tissue with which to replace the urethra. In 1986 Snow placed a tunica vaginalis flap as a wrap to prevent fistula after hypospadias surgery in 20 patients. This tunica vaginalis wrap prevented urethrococutaneous fistulas in all cases. In a pilot study in 1989 Khoury et al applied tunica vaginalis flaps for urethral reconstruction in an experimental setting. In that pilot study the results of circumferential replacement of the urethra with a tubularized flap of tunica vaginalis was examined in 7 rabbits. Contraction of the cremasteric muscle brought with the flap was believed to be re-
sponsible for the voiding difficulties noted in 3 of these 7 animals.

These encouraging results were followed by clinical application of the technique in 1992 by Snow and Cartwright. They used tunica vaginalis on a vascular pedicle as a tube and meatal stenosis developed in all patients. The investigators concluded that tunica vaginalis may be better used as an island onlay type of flap rather than as a circular urethra.

In 1998 Theodorescu et al reported a study comparing functional and histological outcomes after repairing an experimentally induced urethral defect by an onlay flap or a tube flap of tunica vaginalis. All 8 animals that underwent tunica vaginalis tube flap reconstruction experienced neourethra contracture and died of urinary retention. On the other hand, 16 of the 18 animals in the onlay flap group were long-term survivors. They had excellent flap viability and a 100% urethral patency rate after a minimum postoperative observation period of 4 months.

To date published techniques have included graft or flap apposition on the ventral surface of the urethra but the graft often lacks the mechanical support of a fixed bed. This allows it to fold on itself, decreasing the opportunity for neovascularization and the caliber of the reconstructed urethra.

Barbagli et al popularized the concept of dorsal grafts anchored directly to the corpora. This study demonstrated that the dorsal graft procedure using buccal mucosa for bulbar strictures introduces some advantages over traditional ventral onlay graft urethroplasty. Spreading the graft to make use of the tensile strength of the corporeal bodies decreases the risk of graft shrinkage and chordee, the dorsal graft bed avoids the problem of ventral sacculation and interposing the graft between the urethra and corporeal bodies appears to limit fistula formation.

Tunica vaginalis grafts are much easier to harvest than other materials and their application is more rapid. In addition, the donor site is near and the tissue is abundant. Use of the tunica vaginalis graft has the potential to significantly decrease operative time. In hypospadias surgery buccal mucosa harvest increases operative time by 1 hour.

We believe that 1 of the most important properties of the tunica vaginalis graft is the fact that it undergoes conversion into uroepithelium when placed in the urinary tract. The transformation of a monostratified epithelium into a urinary pseudostratified epithelium must be explained because the digestive and urinary tracts embryologically originate from the endoderm.

The explanation of our good results is probably that dorsal placement of the graft enhances graft immobilization and the direct blood supply from the corpora perforating the vessels was probably responsible for good graft incorporation. The results of the current study are encouraging and they support the use of tunica vaginalis grafts for clinical urethral replacement. Before the clinical use of this technique some questions must be clarified. Although the urethral defects corrected were small (1.0 × 1.0 cm), almost 30% of the entire rabbit urethral circumference was replaced. It is uncertain whether larger defects could be corrected with tunica vaginalis. To our knowledge the maximum area of defect that could be corrected with adequate incorporation and without graft contracture is unknown. On the other hand, we recog-
nize that the weakness of our study was the lack of a control group (sham procedure). Experiments at our laboratory are currently in progress to address these questions.

CONCLUSIONS

Tunica vaginalis urethroplasty has good characteristics for tissue handling and urethral function, and it may be considered for clinical use in difficult cases. In the current study we observed that a tunica vaginalis graft can be a successful urethral substitute in the animal model.

REFERENCES